“Edge Effect” of $^{32}$P Radioactive Stents Is Caused by the Combination of Chronic Stent Injury and Radioactive Dose Falloff

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Background—Radioactive stents have been reported to reduce in-stent neointimal thickening. An unexpected increase in neointimal response was observed, however, at the stent-to-artery transitions, the so-called “edge effect.” To investigate the factors involved in this edge effect, we studied stents with 1 radioactive half and 1 regular nonradioactive half, thereby creating a midstenst radioactive dose-falloff zone next to a nonradioactive stent-artery transition at one side and a radioactive stent-artery transition at the other side.

Methods and Results—Half-radioactive stents (n=20) and nonradioactive control stents (n=10) were implanted in the coronary arteries of Yucatan micropigs. Animals received aspirin and clopidogrel as antithrombotics. After 4 weeks, a significant midstenst stenosis was observed by angiography in the half-radioactive stents. Two animals died suddenly because of coronary occlusion at this mid zone at 8 and 10 weeks. At 12-week follow-up angiography, intravascular ultrasound and histomorphometry showed a significant neointimal thickening at the midstenst dose-falloff zone of the half-radioactive stents, but not at the stent-to-artery transitions at both extremities. Such a midstenst response (mean angiographic late loss 1.0 mm) was not observed in the nonradioactive stents (mean loss 0.4 to 0.6 mm; $P<0.01$).

Conclusions—The edge effect of high-dose radioactive stents in porcine coronary arteries is associated with the combination of stent injury and radioactive dose falloff. (Circulation. 2001;104:2236-2241.)

Key Words: stents ■ radioisotopes ■ angioplasty ■ restenosis

The use of stents has markedly reduced the rate of restenosis after percutaneous coronary interventions.1,2 To reduce the restenosis rate even further, endovascular radiation therapy with line sources3–5 or radioactive stents6–8 was introduced. Although both methods proved very effective in reducing restenosis in the irradiated area, significant new disease was introduced at the edges of the treated lesions, particularly with radioactive stents.9–12 There is only anecdotal evidence of the substrate of this edge effect.13 Possible explanations for this edge phenomenon are the stimulation of tissue proliferation14,15 or excessive extracellular matrix16,17 by low-dose irradiation, by mechanical injury,18–20 or by a combination of both. The latter has been described in clinical endovascular radiotherapy as geographic miss.21 Therefore, we performed an experimental study that aimed to discriminate between these factors (radiation dose falloff, dose falloff plus injury, or injury per se) by using specially designed stents made radioactive over half of their length.

Methods

Animal Preparation

Experiments were performed in 10 nonatherosclerotic adult female Yucatan micropigs (20 to 30 kg). The protocol was approved by the Committee on Experimental Animals of Erasmus Medical Center Rotterdam. The day before the procedure, antiplatelet prophylaxis was started with 150 mg clopidogrel (Plavix, Sanofi) and 300 mg aspirin orally. After an overnight fast, the animals were sedated with 20 mg/kg ketamine hydrochloride. Anesthesia was induced with 11 mg/kg thiopental. Anesthesia was maintained with a mixture of oxygen and nitrous oxide (1:2, vol/vol). Intramuscular antibiotic prophylaxis was administered with 100 mg procaine-benzylpenicillin and 250 mg streptomycin. Under sterile conditions, an introduction sheath was placed in the left carotid artery, and 5000 IU heparin was administered. A guiding catheter was advanced to the ascending aorta. Activated partial thromboplastin time was measured at regular intervals and kept at >3 times normal values. After measurement of arterial blood pressure, heart rate, and blood gases, coronary angiography was performed with a high-resolution catheter angiography system (Cardiovision, Toshiba, Japan).
performed with iomeprol (Iomeron 370, Bracco-Byk) as contrast agent.

**Half-Radioactive Multi-Link Stent**

Regular 18-mm-long Multi-Link Duet stents (Guidant) were made radioactive with $^{32}$P ($\beta$-emitter, half-life 14 days) over half of their length in the Forschungszentrum Karlsruhe GmbH, Germany. This yielded 1 radioactive half of 0.9 mCi/mm stent length (range 7.2 to 9.0 mCi/9 mm) and 1 nonradioactive half (Figure 1). Stents were crimped onto dedicated balloons (3.0 mm in diameter) designed with so-called short transitional edge protection (STEP) technology to limit balloon-induced damage outside the stented segment (Figure 2). Half-radioactive stents with the radioactive part directed distally or proximally on the balloons, as well as nonradioactive control stents, were provided sterile, covered by a polymeric shielding, and encoded to allow for random implantation.

**Stent Implantation and Quantitative Coronary Angiography**

A segment with a diameter of 2.7 to 3.0 mm was selected in each coronary artery by use of quantitative coronary angiography (QCA) (CAAS II, PIE Medical). Thereafter, 2 half-radioactive stents (1 hot-distal and 1 hot-proximal) and 1 control stent per animal were randomly implanted in different vessels at 8 atm balloon inflation pressure, aiming at a balloon-to-artery ratio of 1.1:1. After repeat angiography of the stented coronary arteries, the catheter and introducer sheath were removed, and the arteriotomy and the skin were closed. Animals received 300 mg aspirin and 75 mg clopidogrel daily during follow-up. After 4 and 12 weeks, QCA was repeated in the same projection.

**3D Intracoronary Ultrasound Analysis**

Intravascular ultrasound (IVUS) image acquisition was performed at 12-week follow-up with a 30-MHz mechanical system (ClearView, CVIS, Boston Scientific Corp). Motorized catheter pullback was ECG-gated (peak of the R wave) to eliminate motion artifacts (EchoScan, Tomtec). 3D volumetric lumen volume and neointimal volume were assessed with a semiautomated contour detection program. Data are given as volumes normalized to 1 mm stent length (mm$^3$/mm) to allow for direct comparison of zones of different lengths.

**Histomorphometry**

The coronary arteries were pressure-fixed in situ and processed for microscopy as described. After $\geq 7$ half-lives of 14 days, the embedded stents were cut into equal longitudinal halves after alignment under fluoroscopic control. Thereafter, the specimens were placed on radiographic film for 48 hours to allow identification of the radioactive part and the individual radioactive struts (Figure 3).

The neointima on top of the individual stent struts was measured on en-bloc toluidine blue–stained specimens with a microscopy image analysis system (Impak C, Clemex Technologies). The distance between the endothelial lining and the stent strut or internal elastic lamina was taken as the thickness of the intima.

After completion of morphometric analysis, transverse thin sections were cut for qualitative histological examination. Hematoxylin-eosin was used as routine stain, and resorcin-fuchsin was used as elastin stain.

**Definition of Subsegments**

The stented vessel was divided into subsegments that were defined as follows (the stent spanned a distance from 0 to 18 mm, Figure 1): Reference zone: proximal ($\leq 2.5$ to $2.3$ mm) or distal ($21$ to $23$ mm) vessel segment adjacent to the stent and not affected by radiation or balloon injury. Hot transition zone: zone between radioactive half and reference zone (17 to 21 mm). Hot zone: radioactive stent segment with full dose (10 to 16 mm). Mid zone: zone connecting nonradioactive and radioactive stent half (7 to 10 mm). Cold zone: nonradioactive segment within the stent (2 to 6 mm). Cold transition zone: between nonradioactive half and reference zone ($\leq 3$ to $1$ mm).

![Figure 1. Schematic of stent radioactivity. Colored lines at radioactive stent half are cumulative isodose lines at a distance of 0.1 (black), 0.5 (pink), and 1.0 (yellow) mm from stent struts over a period of 12 weeks.](image1)

![Figure 2. Detail of delivery balloon system (STEP technology), which was designed to prevent balloon-induced damage outside stent-covered area (magnification ×20).](image2)

![Figure 3. Explanted stents were embedded in plastic, cut longitudinally (bottom), and placed on radiographic film for 48 hours. After processing of exposed film (top), radioactive half and individual radioactive struts of stents could be identified (magnification ×9).](image3)
Statistical Analysis
Data were expressed as mean±SD. Angiographic data were analyzed by 2-way repeated-measures ANOVA followed by post hoc Dunnett’s test. Histological data were analyzed by 2-way ANOVA and Dunnett’s test. For these parameters, a value of *P<0.05 was considered statistically significant, because Dunnett’s test corrects for repeated testing. IVUS data were analyzed by 2-way ANOVA and unpaired *t* test. Because of repeated comparisons, a value of *P<0.01 was considered statistically significant. Sigmastat and SPSS statistical software were used.

Results
Stent Implantation and Follow-Up
Stents were placed in 10 animals. One animal died at the end of the procedure of respiratory insufficiency. Final analysis was therefore performed in 9 animals.

At 4 weeks, angiography showed a reduction in lumen at the mid zone in all half-radioactive stents, but not in control stents (Figure 4). Two animals were killed to examine this phenomenon. Between 4 and 12 weeks, 2 animals died suddenly. Postmortem examination showed a thrombotic occlusion at the narrowed mid zone of the half-radioactive stent in the LAD. Five surviving animals remained for angiographic, IVUS, and histological examination at 12 weeks.

Radioactivity of Stents After Explantation
Exposure of radiochromic film by the embedded stent specimen revealed the activity of the individual stent struts (Figure 3). Typically, per half-radioactive stent, 7 struts per longitudinal cross section were identified as hot, and the remaining 7 struts were identified as cold.

QCA Measurements
QCA showed similar lumen diameters of the arteries with half-radioactive or control stents at baseline (Table). Average balloon size was 2.8±0.1 mm, and balloon-to-artery ratio was 1.1±0.1. After stenting, the vessels measured 2.8±0.1 mm, and no differences between groups were observed.

At 4 weeks, both types of half-radioactive stents showed a significantly smaller lumen diameter of 1.9 mm in the mid zone versus 2.4 mm in the cold and hot parts of the same stents (*P<0.05). The mean diameter of the control stents was 2.3±0.3 mm, and in the midstent zone, 2.1±0.4 mm (*P=NS).

Control stents showed no change in lumen diameter between 4- and 12-week data (2.2±0.4 mm). In the half-radioactive stents, however, a significant further decline of diameter at the mid zone could be observed. For the “proximal-hot” and the “distal-hot” stents, mid-zone diameters were 1.5±0.3 mm and 1.5±0.9 mm, respectively (*P<0.05 versus control).

3D IVUS
IVUS examination at 12 weeks showed no difference in neointimal volume of the control stent (3.2±3.5 mm³/mm stent), cold zones (4.5±3.9 mm³/mm), and hot zones (2.0±1.3 mm³/mm). At the mid zones of the half-radioactive stents, however, a significant increase in neointimal volume was observed (6.0±3.5 mm³/mm; *P<0.01).

Histopathological Examination
Macroscopy
The half-radioactive stents, but not the control stents, explanted after 4 and 12 weeks all demonstrated narrowing at the mid zone (Figure 5). Specimens retrieved from animals that died suddenly showed thrombotic occlusion at narrowed mid zones of LAD stents.

Microscopy
Both control stents and cold zones of the half-radioactive stents showed the typical appearance of a stented normal artery: mild to moderate intimal thickening consisting of smooth muscle cells (SMCs) in extracellular matrix with

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<th>Mean Angiographic Diameter of Arteries Receiving Stents With the Proximal Half-Radioactive, Distal Half-Radioactive, and Control Stents</th>
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Prox indicates proximal; dist, distal. Per group, the data of 3 zones within the stents (hot zone, mid zone, and cold zone) are shown. Values are in millimeters (mean±SD).

*P<0.05 mid zone vs cold and hot zone.
†P<0.05 vs control.
sparse inflammatory cells and covered by continuous endothelium (Figure 6A).

The mid zone of the half-radioactive stents was characterized by tissue appearing in a specific order (Figure 6B). From cold to hot: (1) enlarged cells with nuclear atypia (as previously described in association with radiotherapy\textsuperscript{24}); (2) a steep increase in neointima containing SMCs in disarray within abundant extracellular matrix, dispersed macrophage foam cells, and amorphous proteinaceous material (edema) infiltrated by leukocytes. Endothelialization and neointimal cellularity decreased in parallel toward the hot zone; and (3) a sharp decrease in intimal thickness with a concomitant change to immature granulation tissue after reaching the first hot stent strut.

The hot zone generally showed immature granulation tissue of variable thickness containing amorphous proteinaceous material with few endothelial cells and a diffuse inflammatory response (Figure 6C). The intima-media border zone contained areas with barely organized thrombotic material (especially overlying stent struts).

The hot transition zone showed asymmetrical intimal thickening with incomplete endothelialization and characteristic pathological features: barely organized thrombotic material overlying the stent struts and the intima-media border zone, foam cells in the intima-media border zone, proteinaceous material infiltrated by leukocytes that composed the body of the intima, arborizing SMCs within an abundant extracellular matrix that composed the body of the intima and luminal border zone, and hypertrophic cells with nuclear atypia scattered throughout the intima and media. Normal vascular architecture returned in both control and half-radioactive stents within 2 mm from the stent edges.

**Morphometry**

Comparison of the mean neointimal thickness over the struts in the cold zone (struts 2 to 5) and the hot zone (struts 10 to 13) with the mid zone struts (struts 7 and 8) showed a significantly larger amount of neointima in the mid zone ($P<0.05$; Figure 7). Also, the difference between the maximal neointimal thickness of the half-radioactive mid zones (0.65±0.06 mm) and the control stent mid zones (0.40±0.08 mm) was significant ($P<0.05$).

**Discussion**

Radioactive Stents Reduce Restenosis but Are Susceptible to Edge Renarrowing

In-stent restenosis is the main limitation of coronary stenting and is caused predominantly by neointimal hyperplasia.\textsuperscript{18–20,25} Stud-
ies with radioactive $^{32}$P stents showed that tissue response within the stent was markedly reduced but revealed a significant remanoring at the edges of the implant. IVUS studies in stented arteries treated with catheter-based brachytherapy demonstrated negative remodeling and neointimal thickening at the edges of the irradiated zone. In the absence of geographic miss, however, stent edge effects were rare. In radioactive stents, however, the incidence of edge restenosis was considerably higher than after catheter-based brachytherapy, especially at doses that reduced in-stent hyperplasia. The present study was designed to gain insight into the mechanism of this edge effect. To that purpose, we studied different configurations of stents and $\beta$-radiation in a half-radioactive stent model. This stent on the STEP balloon allowed us to compare the tissue responses to stent-induced injury, full-dose radiation, radiation falloff, and their combination at 3 distinct transition zones.

Main Findings
In the present study, a moderate reduction of lumen diameter in the control, nonradioactive stents was observed, which was evenly distributed over the length of the stent. In contrast, the half-radioactive stents showed a maximal reduction of lumen diameter at the mid zone. This increased tissue response at the midzone exceeded that at the stent edges and demonstrated distinct histopathological features, such as an increased amount of extracellular material and thrombus, features recently described in a human case report. The mid-zone hyperplasia coincided with the presence of the stent and a sharply decreasing radioactive dose level. Each of these can promote cellular proliferation. The stent does so by inflicting chronic injury, causing inflammation and tissue proliferation. Low-dose radiation ($\pm 2$ Gy) has been shown to potentiate cellular metabolic activities and immunological responses in various cells of mesodermal origin. Furthermore, experimental studies of endovascular brachytherapy have shown that relatively low doses ($\pm 10$ Gy) caused a paradoxical increase in tissue response, whereas higher doses proved antiproliferative. In a model of concanavalin-induced proliferation, low-dose radiation enhanced the tissue response. Combining the chronic mechanical irritation by the stent with low-dose radiation also had an additive effect on tissue proliferation in the present study. These findings suggest that low-dose radiation catalyzes the tissue response to pro-proliferative factors. To the best of our knowledge, low-dose radiation alone was never able to induce such a degree of tissue proliferation in normal tissue. The fact that significant neointima was not observed at the transition from hot stent half to artery underscores this.

Our results indicate that edge effects of radioactive stents may be avoided by limiting arterial trauma at the edges of the stent combined with measures to effectively irradiate the first 2 to 3 mm outside the stent extremities. The former could be done by using dedicated delivery systems or by manufacturing thinner and more flexible stent edges. The latter might be achieved by use of more penetrating radiation qualities, such as $\gamma$-radiation.

Conclusions
This study demonstrates that the combination of radioactive dose falloff and the presence of stent material in the artery wall may be responsible for the edge restenosis that limits the efficacy of radioactive stents in the clinical setting. Further studies on the relationship between radiation dose and tissue response may enhance our understanding of the balance between tissue radiosensitivity, arterial injury, and effective radiation dose.

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References
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