Long-Term Effects of Intracoronary β-Radiation in Balloon- and Stent-Injured Porcine Coronary Arteries

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Background—The data on the long-term safety and efficacy of intracoronary β-radiation in animal models are limited. Methods and Results—A total of 30 coronary arteries in 15 swine were subjected to balloon or stent injury followed by β-radiation from a centered 32P source (2000 cGy to 1 mm beyond lumen surface) or a sham radiation procedure. The animals received aspirin for 6 months and ticlopidine for 30 days. Five of the 10 animals subjected to radiation died (at 5 days, 7 days, 3 months [n=2], and 4 months) as a result of layered, occlusive thrombus at the intervention site (3 stent and 2 balloon injury sites). No deaths occurred in the control group. In the surviving animals, balloon-injured and irradiated vessels showed a trend toward larger lumens than controls (2.15±0.17 versus 1.80±0.08 mm2, P=0.06) and larger external elastic lamina areas (3.32±0.21 versus 2.62±0.10 mm2, P=0.003). In the stent-injured vessels from surviving animals, lumen, neointimal, and external elastic lamina areas were 3.58±0.33, 3.16±0.35, and 8.12±0.42 mm2 for irradiated vessel segments; these values were not different from those in controls (3.21±0.15, 2.84±0.27, and 7.76±0.28 mm2, respectively). Histologically, healing was complete in most survivors, although intramural fibrin and hemorrhage were occasionally seen.

Conclusion—In the long-term (6 month) porcine model of restenosis, the inhibition by intracoronary β-radiotherapy of the neointimal formation that is known to be present at 1 month is not sustained. This lack of effect on neointimal formation after balloon and stent arterial injury is accompanied by subacute and late thrombosis that leads to cardiac death on a background of continuous aspirin but relatively brief ticlopidine treatment. (Circulation. 2001;103:2108-2113.)

Key Words angioplasty ■ stents ■ restenosis ■ radiotherapy

Restenosis remains a formidable problem after percutaneous coronary intervention techniques. Intracoronary radiotherapy has emerged as a promising therapy to inhibit the restenotic process. Animal investigations with both γ and β intracoronary radiotherapy have shown profound inhibition of neointimal proliferation at 2 to 4 weeks after balloon or stent injury. On the basis of these studies, numerous clinical trials have been undertaken. Results of early clinical investigations have indicated a significant reduction in angiographic and clinical measures of target site restenosis in patients receiving intracoronary radiotherapy who are followed for 6 to 9 months.

The long-term effects of intracoronary radiation, however, are uncertain. Although the results of 1-month animal studies seem to correspond reasonably well with findings at 6 months in humans, long-term animal studies, which are potential predictors of late results in humans, are scarce. Further, only limited information regarding the long-term follow-up of humans receiving radiotherapy is available. Given the paucity of the long-term animal and human data, the goal of the present study was to examine the long-term effects of intracoronary β-radiotherapy 6 months after initial balloon and stent injury followed by intracoronary 32P radiolabel media for the porcine coronary artery restenosis model. Specifically, the following issues were addressed: (1) the evolution of known “early” histopathologic changes (fibrin exudate, hemorrhage, inflammation, and fibrosis), (2) the possible delay or inhibition of the vessel healing process, and (3) the persistence of the early (1 month) efficacy (inhibition of neointima).

Methods

Coronary Vessel Injury

All animal treatment and care conformed to the National Institute of Health’s Guidelines for Care and Use of Laboratory Animals. The
protocol was also approved by the Animal Protocol Review Committee of Baylor College of Medicine. Yucatan mini-swine of both sexes that were 3 months old and weighed ~25 to 30 kg were used. Ticlopidine 500 mg and aspirin 325 mg were started 3 days before the initial procedure. Aspirin was continued until euthanization at 6 months, and ticlopidine was administered for 28 days.

The swine were intubated and then allowed to breathe freely with 2% halothane and oxygen-enriched room air. Through a carotid approach, coronary arteriography and intravascular ultrasound (IVUS) were performed. Of the 3 major coronary vessels (right coronary artery, left anterior descending artery, and circumflex artery), 2 were selected for injury and irradiation. In each animal, 1 artery was injured with a balloon oversized 1.2 to 1.3 times the arterial diameter, and one artery received a stent oversized 1.1 times the arterial diameter.

**Radiation Treatment**

The 15 animals were divided into 2 groups representing radiation (n=10) and control (n=5). In each animal, 1 artery was balloon-injured and 1 artery received an oversized stent. Arteries in the radiation group received 2000 cGy delivered to 1 mm beyond the lumen surface after balloon or stent injury at dose rates <50 cGy/s, as based on previous experience. The control group received simulated treatments with no radiation using a dummy wire.

The details of the intracoronary radiotherapy procedure were described previously. The system included an automated after-loader (Guidant-modified Varian unit, model VariSource 2000/A), a centering catheter (Cordis), and a 27-mm-long 32P active source wire as well as an inactive wire. The centering balloon had a 27-mm-long spiral balloon, centered, closed-end lumen for source wire travel and a distal short rapid exchange tip. Its size was selected to be within ±0.3 mm of the IVUS-measured predilation vessel diameters to avoid further damage to the arterial wall.

The simulation wire was advanced first to validate the position within the target zone under fluoroscopy and to correct it when necessary; it was then followed by the active wire, which had its position confirmed fluoroscopically. The dwell time was calculated based on the current source activity and IVUS-defined vessel segment dimensions to yield a total dose of 2000 cGy to 1 mm beyond lumen surface.

**Euthanization, Histology, and Morphometry**

After 6 months, angiographic and IVUS assessment were performed. The animals were then exsanguinated under halothane overdose, and the heart was rapidly excised and perfusion-fixed. The balloon-injured arteries were excised from the hearts and sectioned into 2- to 3-mm slices. Proximal and distal sections that were obviously remote from the injury site were discarded, and the remaining sections (between 10 and 18), were embedded in paraffin and stained with a combination of Masson trichrome and Verhoeff elastic fiber stain. The slides were examined qualitatively and subjected to computer-assisted morphometric analysis. The tracings of the vessel structures and IVUS-defined vessel segment dimensions were used to reproduce the location of these cross-sections for pair-wise comparison.

**Statistical Analysis**

In all sections showing qualitative signs of radiation (adventitial fibrosis, persistent fibrin, or medial thinning), injury (internal elastic lamina tear, media rupture, or neointimal formation), or both, the cross-sectional areas within the lumen (lumen area), internal elastic lamina, and external elastic lamina (EEL) were measured. Mean values of all analyzed sections were then averaged for the radiation and control groups, respectively. Continuous variables are presented as mean±SEM. A t test was used to compare morphometric and IVUS variables between the radiation and control groups using the SigmaStat 2.0 software program (SPSS Inc). P<0.05 was considered significant.

**Results**

Fifteen animals received balloon and stent injuries in each of 2 coronary arteries. Subsequently, 10 were subjected to intracoronary radiation and 5 to sham procedures (controls). Five of the 10 irradiated animals died prematurely (ie, before the intended follow-up of 6 months). All of the deaths were sudden, and they occurred 5 days, 1 week, 3 months (2 animals), and 4 months after the index procedure. Histopathologic analysis of the intervention sites in these animals revealed the presence of occlusive thrombi that were believed to be the cause of the sudden deaths. In each of these animals, the occlusion occurred at the site of the stent (3 animals; Figure 1A) or balloon (2 animals; Figure 1B) injury. The appearance of these occlusive thrombi was heterogeneous and multilayered, suggesting that the deposition of the thrombotic material occurred in a step-wise fashion over a prolonged period of time, culminating in the fatal occlusion (Figure 1).

The 5 surviving animals subjected to intracoronary radiation were euthanized at 6 months and yielded 5 balloon-injured and 5 stent-injured arteries for morphometric analysis. The arteries of most surviving animals showed generally appropriate healing (as assessed by high magnification light microscopy) of the vessel wall with a varying degree of neointimal formation, both in the balloon injury sites and in the stented segments (Figures 2B and 3B). However, residual fibrin deposition and intramural hemorrhage, suggesting incomplete healing, were infrequently observed in the irradiated arteries (Figure 3C). In rare sections, residual luminal thrombus was found (Figure 3D). No signs of persistent inflammation were observed. Adventitial fibrosis was present, but it did not seem to progress beyond the amount commonly observed at 4 weeks.

All 5 control animals survived until euthanization. Qualitative light microscopic analysis revealed complete healing of the intervention sites, with no residual thrombi or fibrin deposition in either the balloon-injured or the stent-injured vessels. Neointimal proliferation was abundant in all control vessels (Figures 2A and 3A).
Morphometry

Summaries of morphometric measurements are shown in Table 1 (balloon-injured vessels) and Table 2 (stent-injured vessels).

Balloon-Injured Vessels

The comparison between irradiated balloon-injured and control arteries revealed a trend toward larger lumens (2.15±0.17 mm$^2$ versus 1.80±0.08 mm$^2$, $P=0.06$) and larger EEL areas (3.32±0.2 mm$^2$ versus 2.62±0.1 mm$^2$, $P<0.05$) in the irradiated group compared with the control group. Neointimal area and percent area stenosis in the irradiated arteries did not differ significantly from the control vessels.

Stent-Injured Vessels

The stent cross-sectional areas in the irradiated arteries were not significantly larger than those in control vessels (6.74±0.4 mm$^2$ versus 6.05±0.2 mm$^2$, $P=NS$). Lumen and EEL areas in the irradiated arteries were essentially similar to those in the control group. Neointimal areas in the arteries subjected to radiation showed a trend toward larger values when compared with controls. The percent area stenosis was nearly identical for both groups.

IVUS

The data are presented in linear graphs showing changes in lumen cross-sectional areas, as measured by IVUS before injury and at euthanization (Figure 4). In the balloon-injured arteries, minimal changes in lumen area were noted over the follow-up period, both for irradiated and control arteries, which did not differ from each other in that respect (Figure 4A).

In the stent-injured arteries (Figure 4B), mild lumen loss was observed for both irradiated and control arteries. Again, there were no differences between the groups. The IVUS data correlated well with the morphometric findings described above.

Discussion

An extensive body of experimental evidence has established the effectiveness of intracoronary radiotherapy in inhibiting restenosis. The efficacy of this therapeutic modality has been demonstrated for both $\gamma$ and $\beta$ sources. These experimental studies have generally used the well-established porcine coronary artery model of restenosis, in which neointimal formation is evident within 4 weeks after overstretch balloon or stent injury to the coronary arteries. The histological appearance of porcine coronary arteries at 4 weeks after such injury closely simulates the histological appearance of human restenosis at 3 to 6 months. The ability of intracoronary radiotherapy to dramatically inhibit this early proliferative response to arterial injury in the pig has been consistent and reproducible by multiple investigative groups.

In sharp contrast to the plethora of studies in this 1-month animal model, there is a paucity of data regarding longer term results in the same animal model. Wakman et al and Wiedermann et al reported a sustained efficacy of intracoronary radiotherapy in inhibiting neointima formation after 6 months. However, these beneficial long-term studies, com-

Figure 1. Histological sections of arteries from animals that died prematurely (magnification, 20×). A, Sudden death after 4 months. Stented artery with multilayered (arrows) occlusive fatal thrombus in absence of neointima. B, Sudden death after 3 months. Total thrombotic occlusion at site of balloon injury in nearly complete absence of neointima. Two distinct layers of thrombus (arrows) are seen.

Figure 2. Histological sections of stented arteries after 6 months of follow-up (magnification 20×). A, Control artery with abundant neointima. B, Irradiated artery with optimal result of radiation: reduced neointimal formation but complete neointimal coverage of stent and no luminal thrombus.
prising 13 and 11 arteries, respectively, addressed balloon injury only, used only γ-radiation with 192Ir, and were confined to the left coronary artery. More comprehensive longer term animal studies that include stenting might be helpful in assessing the long-term potential of intracoronary radiotherapy in humans. Further, no study to date has described the long-term effects of β-radiation. Also, only limited information regarding the long-term follow-up of humans receiving radiotherapy is available.13

In our study, 5 of 10 animals given intracoronary radiotherapy died before reaching the 6-month study end point. No premature deaths occurred among the 5 animals that received the same injury without intracoronary radiotherapy. Those animals that died prematurely exhibited a similar histopathology, consisting of occlusive thrombosis at the site of intervention and radiotherapy. The thrombosis appeared multilayered and heterogeneous, indicating thrombus deposition in a step-wise fashion over an extended period of time rather than a single thrombotic event.

In 3 of the 5 animals that died prematurely, the stented segment was the culprit for thrombotic occlusion. The degree of neointimal formation in these arteries was mild to moderate, but in no instance was it severe enough to account for vessel occlusion and the fatal ischemic outcome. In some specimens (Figure 1), the inhibition of neointima formation was substantial and dramatic, suggesting perhaps that the inhibition of some “protective” neointima left a residual nidus for mural thrombus with subsequent progression to total occlusion.

The surviving animals manifested histopathologic features that are both encouraging and disappointing for the long-term therapeutic potential of intracoronary radiotherapy. The arteries subjected to balloon-only injury at 6 months showed a trend toward larger lumens in those animals that received radiotherapy. This long-term beneficial effect, however, could not be attributed to a durable inhibition of neointima, because neointimal area at 6 months was similar in radiotherapy-treated and control animals. Instead, dilatation of the artery (increased EEL area) or positive remodeling was observed on morphometry. Positive remodeling has been suggested to be an important factor in the beneficial action of intracoronary radiotherapy in the prevention of restenosis on the basis of early human data analyzed by both angiography21 and IVUS.22

Stent-injured arteries, however, responded in a different manner than balloon-injured arteries. The morphometric and IVUS measurements were larger in this group compared with their balloon-injured counterparts due to the oversized stent.
which is commensurate with the increased injury induced by stent placement. Neointimal area was much larger in stent-injured arteries than in balloon-injured arteries, and there were no significant differences between radiotherapy and control arteries, suggesting a lack of sustained inhibition by radiotherapy of neointimal formation in this long-term model.

On a positive note, most surviving animals showed complete healing of the induced arterial injury with neointimal coverage of the sites of visible stent or balloon-injury when assessed with high-magnification light microscopy. In addition, there was no evidence of residual inflammation, as has been noted in arterial specimens obtained 4 weeks after injury.4,8,10

A small amount of luminal thrombosis seems to be inherent with the arterial injury itself.23,24 The incidence of this thrombosis seems to increase when the intracoronary radiation follows the injury.25 Endovascular radiation after balloon angioplasty also results in delayed resolution of intramural hemorrhage and an increase in platelet recruitment at 1 month.26 However, a hypothesis might be offered that “healing” of the arteries subjected to radiation can and will occur if the step-wise layering of thrombus can be inhibited or avoided. The layering of thrombus promotes 2 activities that interfere with the healing of the arterial wound. First, it retards neointima formation and reendothelialization of the injury zone by creating a new layer and surface for endothelial repopulation. Second, the primary thrombus itself is thrombogenic, encouraging additional new thrombus to form, reducing the lumen further and rendering the reendothelialization effort futile. Unfortunately, the process can conclude with complete closure of the vessel. Eventually, delayed reendothelialization may permit more aggressive neointimal proliferation,27 which might contribute to the lack of effective inhibition of neointimal growth in the long-term porcine coronary artery.

### Limitations
A small sample size makes detection of quantitative differences difficult. The relevance of the 6-month porcine coronary artery model to the long-term outcome in human coronary arteries is not established. Several fundamental differences exist. First, the animals are young (3 months) and in an active stage of growth when subjected to coronary artery injury and radiotherapy. In contrast, the average age in human clinical trials exceeds 60 years, when normal active growth has long ceased. Second, the porcine coronary artery is healthy when subjected to injury and radiotherapy. Quite the opposite is true of human arteries undergoing angioplasty. For these reasons, the long-term response of human coronary arteries to angioplasty, stent deployment, and radiation may be very different than that observed in the porcine model we used in this study.

### Clinical Implications
The presence of occlusive thrombi in the injured and radiated segments of the coronary arteries with ticlopidine limited to 1 month would indicate the need for more effective long-term antiplatelet or anticoagulant therapy to offset the thrombotic potential of arteries undergoing intervention followed by radiotherapy.28,29 Although late thrombosis in human radiotherapy clinical trials has occurred predominately, but not

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**TABLE 1. Summary of Morphometric Data for Balloon-Injured Arteries**

<table>
<thead>
<tr>
<th></th>
<th>ICR (n=5)</th>
<th>Control (n=5)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lumen area, mm²</td>
<td>2.15±0.17</td>
<td>1.80±0.08</td>
<td>0.06</td>
</tr>
<tr>
<td>EEL area, mm²</td>
<td>3.32±0.21</td>
<td>2.62±0.1</td>
<td>0.003</td>
</tr>
<tr>
<td>Neointimal area, mm²</td>
<td>0.38±0.05</td>
<td>0.28±0.03</td>
<td>NS</td>
</tr>
<tr>
<td>Percent area stenosis, %</td>
<td>17±2</td>
<td>13±1</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are mean±SEM. ICR indicates intracoronary radiation.

**TABLE 2. Summary of Morphometric Data for Stent-Injured Arteries**

<table>
<thead>
<tr>
<th></th>
<th>ICR (n=5)</th>
<th>Control (n=5)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lumen area, mm²</td>
<td>3.58±0.03</td>
<td>3.21±0.15</td>
<td>NS</td>
</tr>
<tr>
<td>Stent area, mm²</td>
<td>6.74±0.4</td>
<td>6.05±0.2</td>
<td>NS</td>
</tr>
<tr>
<td>EEL area, mm²</td>
<td>8.12±0.42</td>
<td>7.76±0.28</td>
<td>NS</td>
</tr>
<tr>
<td>Neointimal area, mm²</td>
<td>3.16±0.34</td>
<td>2.84±0.27</td>
<td>NS</td>
</tr>
<tr>
<td>Percent area stenosis, %</td>
<td>46±4</td>
<td>47±4</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are mean±SEM. ICR indicates intracoronary radiation.
exclusively, in arteries receiving new stents at the index procedure, the observation that arterial thrombosis also occurred in balloon-injured arteries in this experimental study would suggest that prolonged antiplatelet therapy would potentially benefit all patients undergoing coronary angioplasty and radiotherapy. Finally, the presence of multiaged, layered thrombus rather than homogeneous (single-event) occlusive thrombosis offers hope that sustained drug therapy that inhibits vascular thrombosis will effectively maintain lumen patency in treated arteries.29

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References
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