Effects of Intracoronary Radiation on Thrombosis After Balloon Overstretch Injury in the Porcine Model

Yoram Vodovotz, PhD; Ron Waksman, MD; Won-Ho Kim, MD; Balram Bhargava, MD; Rosanna C. Chan, PhD; Martin Leon, MD

Background—The main complications of PTCA remain thrombosis and restenosis. Recent studies have demonstrated reduction in the neointimal hyperplasia after intracoronary radiation (IR) with doses of 10 to 25 Gy of ionizing radiation delivered by either β- or γ-emitters to injured vessels. The purpose of this study was to examine the effect of ionizing radiation on the thrombosis rate (TR) of injured porcine coronary arteries.

Methods and Results—Thirty-four juvenile swine (63 coronary arteries) were subjected to overstretch balloon injury followed by IR with doses of 0 to 18 Gy of either β- or γ-radiation. Two weeks after treatment, tissue sections were perfusion-fixed, stained with hematoxylin-eosin and Verhoeff–van Gieson’s stain, and analyzed for presence of a thrombus, thrombus morphology, and neointima formation by computer-assisted histomorphometry techniques. Although the overall TR increased dose-dependently from 0 to 18 Gy prescribed dose, luminal thrombi decreased. Thrombus area also decreased with increasing radiation dose, whether assessed at the prescription point or at the luminal surface, which corresponded to decreased intimal area. Furthermore, luminal thrombi present after IR tended to consist mostly of fibrin and thus were less organized than in controls.

Conclusions—These results suggest that IR induces thrombosis but does not necessarily compromise the lumen. Strategies for reducing TR may further decrease intimal area as well as increasing the safety of this therapy. (Circulation. 1999;100:2527-2533.)

Key Words: thrombosis ■ radiation ■ restenosis

Percutaneous transluminal coronary angioplasty (PTCA) has been used successfully to treat atherosclerotic coronary artery disease but has been accompanied by a rate of restenosis of 30% to 50% due to neointimal hyperplasia and unfavorable remodeling.1 One of the parameters considered key for the inflammatory process, which precedes the formation of neointima, is the presence of platelet thrombi.1–3 Although pharmacological therapies have proved to be largely ineffective at reducing restenosis after PTCA in coronary arteries, some therapies using antagonists of the platelet adhesion molecule GP IIa/IIIb have been associated with reduction of thrombotic events—and possibly also neointima—after intervention.1 A promising new avenue for the reduction of restenosis comes from recent animal studies and pilot clinical studies using intracoronary radiation (IR).4 However, it is of primary interest to devise means by which to reduce the attendant complications of this therapeutic modality and to reduce the dose of radiation required to achieve a reduction in restenosis.

Doses of external-beam radiation similar to those used to treat balloon-injured coronary arteries in animal models result in increased thrombosis and delayed wound healing.5,6 There have been anecdotal reports of subacute thrombosis (<30 days) and late thrombosis (>30 days) after IR despite treatment with antithrombotic agents in the feasibility clinical studies.7 This finding is paradoxical, because thrombosis has been linked to increased neointimal hyperplasia,1–3 whereas IR reduces neointimal hyperplasia.4 To address this paradox and to study this phenomenon in detail, we set out to examine the overall thrombosis rate (TR), the luminal thrombosis rate (LT), the nonluminal thrombosis rate (NLT), and the thrombus area (TA) after treatment with doses of IR ranging from 0 to 18 Gy of either β- or γ-radiation prescribed to the adventitia. These issues were addressed in a retrospective study in the porcine model of balloon overstretch injury (BI) at 14 days after treatment. This time point was chosen because previous studies had shown that the efficacy of catheter-based IR at suppressing intima formation at this time point persisted to 6 months8,9 and because the thrombosis observed clinically appeared to be subacute.7

Methods

BI Procedural Details

The animals (51 domestic juvenile swine, weight 30 to 45 kg [Thomas Morris, Inc, Reiserstown, Md]) were subjected to BI in the...
left anterior descending, left circumflex, or right coronary artery (a total of 76 arteries) as described previously.\[^{8,10}\] Briefly, the animals were sedated with a combination of ketamine 25 mg/kg and xylazine 2 mg/kg IM, then intubated and ventilated with oxygen (2 L/min), nitrous oxide (2 L/min), and isoflurane 1% (1.5 L/min) with a Harvard respirator. All animals were pretreated with aspirin 325 mg and ticlopidine 250 mg BID 24 hours before administration of a dummy wire, and in others, no wire was sufficient to deliver the prescribed doses of 0 to 18 Gy (2 to 20 minutes). In some cases, the 0-Gy dose was delivered by administration of a dummy wire, and in others, no wire was administered; no significant quantitative or qualitative differences in extent of injury or thrombosis were observed between these 2 types of arteries (data not shown).

The dosages described above of the various isotopes used in this study were also recalculated to reflect dose at the luminal surface. The calculations of luminal dose were different depending on whether the isotope was delivered via a centering system (\(^{133}\)Xe, \(^{90}\)Y) or a noncentering system (\(^{192}\)Ir, \(^{186/188}\)Re). Essentially, noncentering systems had maximal and minimal doses, whereas the centering systems did not. Consequently, we calculated the luminal dose on the basis of the average of the minimum and maximum doses for \(^{192}\)Ir and \(^{186/188}\)Re and used a simple multiple for \(^{133}\)Xe and \(^{90}\)Y (Table 1).

### Table 1. Calculation of Luminal Dose for the Isotopes Used in These Studies

<table>
<thead>
<tr>
<th>Isotope</th>
<th>Maximum*</th>
<th>Minimum*</th>
<th>Average</th>
<th>Prescribed dose(s), Gy</th>
<th>Luminal dose(s),† Gy</th>
</tr>
</thead>
<tbody>
<tr>
<td>(^{192})Ir</td>
<td>3.0</td>
<td>0.9</td>
<td>1.9</td>
<td>5, 15</td>
<td>9.75, 29</td>
</tr>
<tr>
<td>(^{186/188})Re</td>
<td>10</td>
<td>1.2</td>
<td>5.6</td>
<td>15</td>
<td>84</td>
</tr>
<tr>
<td>(^{133})Xe</td>
<td>2.27</td>
<td>NA</td>
<td>NA</td>
<td>15</td>
<td>150</td>
</tr>
<tr>
<td>(^{90})Y</td>
<td>41</td>
<td>NA</td>
<td>NA</td>
<td>18</td>
<td>41</td>
</tr>
</tbody>
</table>

Dosimetry was performed as described in the Methods. NA indicates not applicable. *Numbers indicate multiples of prescribed doses. †Calculated by multiplying by average of maximum and minimum (\(^{192}\)Ir, \(^{186/188}\)Re) or by multiplying by maximum dose multiplier (\(^{90}\)Y, \(^{133}\)Xe).

### Radiation Procedural Details

After the BI, a closed-end lumen catheter with marker was positioned over the injured site for use to deliver radiation dose. The position of the catheter was checked with fluoroscopy to ensure adequate coverage at both ends of the injured site. The 4 different radiation sources examined as part of this study were \(^{192}\)Ir ribbon from Best Medical (\(n=17\) arteries), \(^{186/188}\)Re wire from Soreq (\(n=10\) arteries), \(^{90}\)Y wire from Schneider (\(n=18\) arteries), and \(^{133}\)Xe gas balloon from Cook Cardiology (\(n=9\) arteries). The first 2 were noncentering \(\eta\) and \(\beta\) sources, respectively, and the remaining 2 were centered \(\beta\) sources. Dosimetric calculations were carried out by use of the TG43 algorithm\[^{11}\] with data generated by Monte Carlo calculations following TG60 recommendations.\[^{12}\] Dose prescription points were those used in previous animal studies, 2 mm from source center for \(^{192}\)Ir ribbon,\[^{8}\] 2 mm from source surface for \(^{186/188}\)Re wire,\[^{13}\] 1 mm from balloon surface for \(^{90}\)Y wire,\[^{14}\] and 0.25 mm from balloon surface for \(^{133}\)Xe gas \[^{15}\] (Table 1). All sources were left in the catheter for a period sufficient to deliver the prescribed doses of 0 to 18 Gy (2 to 20 minutes). In some cases, the 0-Gy dose was delivered by administration of a dummy wire, and in others, no wire was administered; no significant quantitative or qualitative differences in extent of injury or thrombosis were observed between these 2 types of arteries (data not shown).

The positions of the catheter were checked with fluoroscopy to ensure adequate coverage at both ends of the injured site. The 4

### Statistical Methods

Comparisons of TA, IA/FL, TA/VA, and TA/FL between control and irradiated arteries were made by either 1-way ANOVA with the Bonferroni correction for groups whose SD of the means was not statistically different (\(P<0.05\) by Bartlett’s test) or by the Kruskall-Wallis test for groups whose SD of the means was statistically different (\(P<0.05\) by Bartlett’s test). Differences in

### Tissue Analysis Protocol

After an angiogram was obtained under anesthesia, the animals were killed 14 days after radiation or placebo treatment by injection of a euthanasia solution (390 mg/mL pentobarbital sodium, 1% propylene glycol, 29% ethyl alcohol, 2% benzyl alcohol). The hearts were perfusion-fixed, and the injured segments were dissected free from the heart. Serial 2- to 3-mm transverse segments were processed and embedded in paraffin. Cross sections (4 \(\mu\)m) were stained with hematoxylin and eosin (H&E) and Verhoeff–van Gieson elastin (VVG) stain. An experienced observer blinded to the treatment group examined all histological sections. Each specimen was evaluated for the presence of thrombus, neointima formation, and morphological appearance of the cells within the media, adventitia, and neointima.

Histomorphometric analysis was performed on each segment with evidence of medial fracture. The histopathological features were measured by use of a computerized PC-compatible image analysis program (Optimas 6; Optimas, Inc). VVG-stained sections were magnified at \(\times25\), digitized, and stored in a frame-grabber board (DAGE-MTI). Thrombi were identified and designated as luminal or nonluminal (mural), depending on their predominant location in the artery: LTs were those thrombi in which \(>75\%\) of the thrombus was present in the lumen, whereas NLTs were those thrombi in which \(>75\%\) of the thrombus was present either between the media and the adventitia or completely in the adventitia. Area measurements were obtained by tracing the thrombus perimeter (TA, mm\(^2\)), neointima perimeter (intimal area, IA, mm\(^2\)), defined by the borders of the internal elastic lamina, lumen, media, and external elastic lamina), and external elastic lamina (vessel area, VA, mm\(^2\)). The total TA was calculated by summing individual TAs. The arc length of the medial fracture (FL), traced through the neointima from one dissected medial end to the other, was used as a measure of the extent of injury. To correct for extent of injury, the ratios IA/FL and TA/FL were calculated. To correct for thrombosis as a function of vessel size, the ratio TA/FL was calculated.
TRs were analyzed by \( \chi^2 \) analysis. Statistically significant differences between treatment groups were considered to be those with 0.05 vs 0 Gy.

Results

TR and LT

In this retrospective study, we examined 51 animals in which 76 arteries were subjected to BI and subsequently treated as follows: 0 Gy (n=22 arteries), 5 Gy (n=10 arteries), 15 Gy (n=26 arteries), and 18 Gy (n=18 arteries). All arteries were examined 14 days after treatment; no arteries from animals that died prematurely before this time point were analyzed.

We first analyzed the histological incidence of thrombosis (GT) as a function of radiation dose. This initial analysis did not discriminate between LT and NLT, nor did we apply a minimal TA as an inclusion criterion for this analysis. As shown in Figure 1, increased TR was observed with increasing doses of IR from 5 to 18 Gy (0.05 versus 0 Gy). However, when arteries were evaluated for the presence of GT, as opposed to NLT, we found that LT decreased with increasing doses of IR (Figure 1; 0.05).

Representative photomicrographs from these experiments are shown in Figure 2. These photomicrographs demonstrate typical LT (Figure 2, A, C, E, and G) and NLT (Figure 2, B, D, F, and H) in arteries subjected to BI alone (Figure 2, A and B), BI+5 Gy (Figure 2, C and D), BI+15 Gy (Figure 2, E and F), or BI+18 Gy (Figure 2, G and H), respectively. Most LTs observed at 0 and 5 Gy appeared to be acute or subacute, consisting of platelet and/or fibrin deposits that in many cases tended to occlude the vessel by 75% (Figure 2, A and C). The insets in these panels demonstrate the presence of erythrocytes and granulocytes. In contrast, the thrombi present in arteries treated with 15 or 18 Gy tended to be NLTs (mural), consisting of a more organized fibrinous layer of thrombus along the surface of the injured artery (Figure 2, F and H; see also insets). As can be surmised from Figure 1, few LTs were observed at 15 or 18 Gy, and this is represented in Figure 2, E and F. Furthermore, the LTs present at 15 and 18 Gy were immature and consisted mostly of fibrin in a disorganized pattern. In general, these thrombi were acellular, lacking in monocytes, lymphocytes, and granulocytes. An-

giographic analysis confirmed the presence of total occlusions as quantified histomorphometrically (data not shown).

TA and IA

The extent of thrombosis has been reported to affect the extent of restenosis, as can be seen in Figure 3A, the extent of intimal hyperplasia measured as IA corrected for medial FL decreased in a radiation dose–dependent fashion (0.05). These findings are in agreement with previous results from studies that used both \( \beta \)- and \( \gamma \)-emitters. At the same radiation doses, TA also decreased in a dose-dependent fashion (Figure 3A; 0.05). A plot of TA versus IA/FL extracted from these data demonstrated a linear relationship (0.05; Figure 3B).

It may be argued that analyses based on prescribed dose are not correct and that in fact the radiation dose to the luminal surface of the vessel affects thrombosis more directly. The isotopes used in these studies vary greatly with respect to type of radiation produced (\( \beta \) versus \( \gamma \) versus a mixture of the two), delivery system (catheter-based versus balloon-based), and centering versus noncentering. These variations can contribute to greatly differing doses at the luminal surface (Table 1). Accordingly, we reanalyzed TA as a function of luminal dose. As seen in Figure 4, we obtained a relationship similar to that observed with doses calculated at the prescription point.

We next compared TR and TA in arteries subjected to BI followed by IR using isotopes with similar prescription point doses (15 Gy) but with widely different luminal surface doses (133Xe and 186/188Re) compared with an isotope with a higher prescription point dose (18 Gy) but a lower luminal surface dose (60Y; Table 2). In addition, sample sizes were nearly identical in all categories. This analysis demonstrated no significant differences in TR and TA between arteries treated with 133Xe and 186/188Re (Table 2), although both groups exhibited significantly greater TR and significantly lower TA and IA/FL than did animals receiving 0 Gy (Table 2). These similar parameters were observed despite the almost 2-fold difference in dose at the luminal surface (84 versus 150 Gy). Furthermore, the highest TR was observed with 18 Gy of 60Y, a dose that corresponds to only 41 Gy at the luminal surface (Table 1).

Because the vessel size (VA) or extent of injury (indicated by FL) may affect the degree of thrombosis, we plotted TA/VA (Figure 5A) or TA/FL (Figure 5B) as a function of radiation dose. Both these plots were similar to that obtained by plotting TA versus radiation dose (Figures 3 and 4), and both demonstrated statistically significant reduction of TA/VA or TA/FL as a function of radiation dose.

Discussion

We and others have been examining the endovascular administration of ionizing radiation and determined that this therapeutic modality reduces neointimal thickening, at least in part by suppressing the proliferative response and inducing favorable remodeling10,17–19; however, this is the first publication to present data related to the effect of intravascular radiation on thrombosis in the porcine BI model.
Figure 2. Histological appearance of thrombosed arteries subjected to BI followed by IR. Porcine coronary arteries were treated as described in Figure 1 and stained with VVG and are shown at a magnification of ×7.5. White arrows indicate thrombi, which are shown at ×1500 magnification of H&E-stained sections in insets. A, 0 Gy, typical LT. B, 0 Gy, typical mural thrombus. C, 5 Gy, typical LT. D, 5 Gy, typical mural thrombus. E, 15 Gy, typical LT. F, 15 Gy, typical mural thrombus. G, 18 Gy, typical LT. H, 18 Gy, typical mural thrombus.
Previous studies on the vascular effects of external beam radiation have suggested that increased thrombosis is an adverse late healing effect. Our findings suggest that IR is associated only with increased TR, but the percent of LT decreases with increasing IR. This finding would suggest that the healing response is indeed delayed, especially because mural thrombosis may be a feature of early arterial healing. Furthermore, the thrombi present in irradiated arteries tended to be acellular, lacking cells thought to be involved in the healing response to arterial injury (monocytes, lymphocytes, and granulocytes). Decreases in the numbers or functions of macrophages may increase the residence of the thrombus and its components at the injured segment and may delay its organization. Our findings may suggest that the healing of irradiated arteries is delayed, and this may have adverse functional consequences.

However, we also found that TA decreases with increasing IR, whether the dose is calculated at the prescription point in the adventitia or at the luminal surface. Likewise, IA/FL decreases with increasing IR, and plotting IA/FL as a function of TA yielded a perfect correlation (Figure 3B). This finding is in agreement with previous reports of an association between extent of thrombosis (ie, TA) and intimal hyperplasia.

It is well known that the LTs are more important and clinically relevant in the short term, because they may propagate, leading to total occlusion, or may dislodge, leading to distal embolization. Conversely, the organized mural thrombi are not relevant in the short term but may contribute to the initiation and development of atherosclerosis and restenosis. One intriguing finding of these studies is that TA was more closely associated with dose at the prescription point in the adventitia than with luminal surface dose. Table 2 shows that arteries treated with isotopes that have identical doses at the

![Figure 3. Effect of BI followed by IR on TA and IA as a function of dose at adventitial prescription point. Porcine coronary arteries were treated as described in Figure 1, with dose calculated to prescription point as described in Methods. A, Sections of perfusion-fixed arteries were analyzed by computer-aided histomorphometry to measure TA (○) and IA corrected for medial FL (IA/FL; □). Symbols represent mean±SEM of 9 (0 Gy), 4 (5 Gy), 16 (15 Gy), and 16 (8 Gy) thrombosed arteries. B, Plot of mean TA vs mean IA/FL at each radiation dose (0, 5, 15, and 18 Gy); r²=1.00. Symbols represent mean of 9 (0 Gy), 4 (5 Gy), 16 (15 Gy), and 16 (8 Gy) thrombosed arteries. *P<0.05 vs TA at 0 Gy; †P<0.05 vs IA/FL at 0 Gy.

![Figure 4. Effect of BI followed by IR on TA as assessed by dose to luminal surface. TA data described in Figure 3A were replotted as a function of dose to luminal surface. Symbols represent mean±SEM of 9 (0 Gy), 4 (9.75 Gy), 3 (29 Gy), 15 (41 Gy), 7 (84 Gy), and 7 (150 Gy). *P<0.05 vs TA at 0 Gy.

![Table 2. Comparison of Thrombosis and Neointima as a Function of Adventitial Dose or Luminal Surface Dose](http://circ.ahajournals.org/)

<table>
<thead>
<tr>
<th>Isotope Delivery System</th>
<th>Prescribed Dose</th>
<th>Luminal Dose</th>
<th>FL, mm²</th>
<th>IA/FL</th>
<th>TR, %</th>
<th>TA, mm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control NA</td>
<td>0</td>
<td>0</td>
<td>1.98±0.17* (n=27)</td>
<td>0.58±0.06 (n=27)</td>
<td>26</td>
<td>3.06±0.57 (n=7)</td>
</tr>
<tr>
<td>186/188Re Noncentered</td>
<td>15</td>
<td>84</td>
<td>2.34±0.28* (n=11)</td>
<td>0.08±0.06† (n=11)</td>
<td>64</td>
<td>0.61±0.16† (n=7)</td>
</tr>
<tr>
<td>133Xe Centered</td>
<td>15</td>
<td>150</td>
<td>2.15±0.23* (n=12)</td>
<td>0.07±0.04† (n=12)</td>
<td>58</td>
<td>1.01±0.22†‡ (n=7)</td>
</tr>
<tr>
<td>90Y Centered</td>
<td>18</td>
<td>41</td>
<td>3.07±0.23 (n=18)</td>
<td>0.14±0.06† (n=18)</td>
<td>83</td>
<td>1.08±0.23‡§</td>
</tr>
</tbody>
</table>

Porcine coronary arteries were subjected to BI followed by 0 (Control), 15 Gy of IR with 133Xe or 186/188Re, or 18 Gy of 90Y. The animals were killed at 14 days, and sections of perfusion-fixed arteries exhibiting thrombi were analyzed by computer-aided histomorphometry for medial FL (an index of arterial injury), IA corrected for medial FL (IA/FL), overall TR, and TA. Numbers are mean±SEM.

*No statistical differences among the groups.
†P<0.05 vs control.
‡No statistical difference vs 186/188Re.
§No statistical difference vs 186/188Re or 133Xe.
Conclusions
This study demonstrated an effect of intracoronary radiation on thrombus formation and thrombus morphology. Higher doses of radiation were associated with increases in TR, but more often a nonobstructive NLT rather than an LT. In addition, the morphology of thrombi in porcine irradiated arteries appeared to be less organized than the pattern of thrombosis observed in nonirradiated injured arteries. These changes in TR and thrombus pattern after radiation therapy may influence the clinical strategy with antiplatelet therapy in patients undergoing intervention followed by vascular brachytherapy.

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References


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