The Effect of Endovascular Irradiation on Platelet Recruitment at Sites of Balloon Angioplasty in Pig Coronary Arteries

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Background—Endovascular irradiation (EI) inhibits balloon-induced neointima formation in animals and is now in clinical trials for restenosis prevention. However, little is known of the effect of EI on vessel thrombogenicity due to delayed arterial healing. We investigated EI effects on platelet recruitment in pig coronary arteries.

Methods and Results—EI was performed using $^{90}$Sr/Y at 0 Gray (Gy), 15Gy, or 30Gy at 2 mm after balloon overstretch injury. At 1 day, 1 week, and 1 month, platelet recruitment and thrombus formation were assessed using autologous $^{111}$In-oxine-platelet labeling and light and scanning electron microscopy. In balloon-injured nonirradiated vessels, there was complete reendothelialization at 1 month, and platelet recruitment was similar to normal uninjured arteries. In irradiated vessels, scanning electron microscopy showed incomplete reendothelialization at 1 month, and these areas demonstrated attachment of activated platelets. Light microscopy of irradiated coronaries showed adherent partially organized thrombi and incomplete resolution of intramural hemorrhages. There was a significant increase in platelet recruitment at 1 month in arteries receiving EI at 15Gy (5.1±2.8×10⁶, P=0.02) or 30Gy (12.5±9.9×10⁶, P=0.005) compared with nonirradiated controls (2.7±1.5×10⁶); 30Gy was also higher than 15Gy (P=0.05). Platelet recruitment was also increased for 30Gy compared with control at 1 day.

Conclusions—Endovascular irradiation at 15Gy or 30Gy after balloon angioplasty results in incomplete endothelial recovery, impaired resolution of intramural hemorrhage, and a dose-dependent increase in platelet recruitment at 1 month. (Circulation. 2000;101:1087-1090.)

Key Words: balloon angioplasty ■ restenosis ■ radiation ■ blood platelets ■ thrombosis

Restenosis remains the major complication of percutaneous transluminal coronary angioplasty.1,2 Stent implantation initially appeared promising, with rates of around 20% to 30% in so-called “ideal” lesions.3,4 However, with improved stent technology, indications for stenting have broadened, and the restenosis rate has climbed.5–7 More recently, endovascular irradiation (EI) has emerged and progressed into clinical trials.8–11 Although radiation appears to delay postangioplasty arterial healing, little is known about the effects of EI on endothelial recovery or thrombus formation. We investigated the effect of EI on platelet recruitment at various time points after balloon injury in the coronaries of adult pigs.

Methods
All experiments and animal care conformed to National Institutes of Health and American Heart Association guidelines and were approved by the Institutional Animal Care and Use Committee of Emory University. Female and castrated male adult minipigs (Yucatan strain, Lone Star Swine, Seguine, Texas) received balloon overstretch injury of all 3 coronary arteries followed by EI at doses of 15 Gray (Gy) or 30Gy or no EI (0Gy controls), according to constrained randomization. All animals received periprocedural aspirin and heparin but no chronic anticoagulant or antiplatelet agent.

Interventional Procedure
We performed coronary balloon overstretch injury in pigs (balloon-to-artery ratio of 1.2 to 1.3, resulting in medial rupture) followed by $\beta$-radiation, as previously described.12 In the case of control animals (no irradiation), sham treatment was performed using the source delivery catheter. Radiation treatment times were 3 minutes and 17 seconds for 15Gy and 6 minutes and 34 seconds for 30Gy based on the dose rate and dose distribution of the $^{90}$Sr/Y source train measured by the National Institute of Standards and Technology.

Platelet Labeling and Tissue Processing
At serial time points (1, 7, and 28 days) after catheterization, animals were euthanized 2 hours after reinfusion of autologous $^{111}$Indium-oxine-platelet labeling and light and scanning electron microscopy. In balloon-injured nonirradiated vessels, there was complete reendothelialization at 1 month, and platelet recruitment was similar to normal uninjured arteries. In irradiated vessels, scanning electron microscopy showed incomplete reendothelialization at 1 month, and these areas demonstrated attachment of activated platelets. Light microscopy of irradiated coronaries showed adherent partially organized thrombi and incomplete resolution of intramural hemorrhages. There was a significant increase in platelet recruitment at 1 month in arteries receiving EI at 15Gy (5.1±2.8×10⁶, P=0.02) or 30Gy (12.5±9.9×10⁶, P=0.005) compared with nonirradiated controls (2.7±1.5×10⁶); 30Gy was also higher than 15Gy (P=0.05). Platelet recruitment was also increased for 30Gy compared with control at 1 day.

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labeled platelets. The heart was rapidly excised and perfusion-fixed. Arterial segments (2.5 cm) were trimmed of excess tissue, and "In activity was measured; platelet recruitment was determined using methods previously described and expressed as number of platelets/vessel after determination of activity/platelet from simultaneously harvested blood samples. Sections of these segments were fixed in glutaraldehyde and processed using standard techniques to determine vessel, neointimal, and intimal thrombus size, as well as intramural hemorrhage, and healing response. Planimetry was used to examine the effects of radiation and time on platelet recruitment. Follow-up comparisons were made using unpaired t tests, or when assumptions of normal distribution or equal variance were violated, Mann-Whitney rank-sum tests were performed. An alpha level of P<0.05 was considered to indicate a significant treatment effect or between-groups difference.

### Results

Light microscopy demonstrated that all vessels underwent medial rupture to an equivalent degree (FL in Table 1). Control arteries developed fibrous neointima; thrombus was present at 1 week but resorbed by 1 month (Table 1). In contrast, neointima of irradiated arteries (15Gy or 30Gy) at 1 month was almost exclusively inspissated thrombus with little cellular ingrowth (Table 1). Intimal thrombus area in controls, but not in 15- or 30-Gy treated vessels, decreased from 1 week to 1 month.

SEM of control vessels showed that injured areas were completely covered by a monolayer of endothelial-like cells by 1 month with only rare adherent platelets. However, irradiated arteries (15Gy or 30Gy) were not reendothelialized at 1 month and showed an abundance of adherent dendritic platelets, fibrin, and leukocytes (Figure 1). Approximately 25% of 15Gy-treated vessel and 50% of 30Gy-treated vessel surfaces were not endothelialized at 1 month.

Platelet recruitment measured by "In-labeling was significantly increased by radiation treatment (F=7.26, P=0.002). Follow-up comparisons showed 30Gy resulted in a significant increase in platelet recruitment compared with controls at both 1 day (52.2±35.9 versus 13.9±14.5×10⁶, P=0.03) and 1 month (12.5±9.9 versus 2.7±1.5×10⁶, P=0.005), respectively. There was increased platelet recruitment at 1 month for 15Gy compared with control (5.1±2.8 versus 2.7±1.5×10⁶, P=0.02) and for 30Gy compared with 15Gy (12.5±9.9 versus 5.1±2.8×10⁶, P=0.05; Table 1 and Figure 2).

### Discussion

We evaluated the magnitude of platelet recruitment in pig coronary arteries treated with 3 doses of EI (0Gy, 15Gy, and 30Gy) and at 3 time points (1 day, 1 week, and 1 month) after balloon angioplasty. We also performed morphological assessment of these vessels. A dose of 15Gy at 2 mm from the source-center was used because it was within the range of doses used for clinical studies, whereas 30Gy was included to help define dose ranges for biological effects.

Light microscopy demonstrated endovascular irradiation at 15Gy or 30Gy resulted in delayed reendothelialization and incomplete resolution of intramural hemorrhages. This provides support for the concept that radiation delays healing after arterial injury. Mural thrombi were inspissated and showed minimal organization in sections from irradiated vessels with superimposed fresh platelet-rich thrombi. SEM confirmed irradiated arteries were not reendothelialized at 1 month in contrast to controls. The non-reendothelialized areas displayed abundant adherent dendritic platelets and leukocytes.

For quantitation of platelet recruitment, we used autologous "In-oxine platelet labeling, a method applied extensively for studies of arterial thrombosis, including pig
Figure 1. SEM of pig coronary arteries fixed 1 month after balloon angioplasty with or without EI. Left-hand panels, low-magnification survey micrographs; right-hand panels, higher magnification images. a and b, control; c and d, 15Gy; e and f, 30Gy. Note recovery of luminal surface with confluent endothelial-like cells in control but lack of re-endothelialization and adherent activated platelets and leukocytes in irradiated vessels.
coronaries. The labeled platelet population remains functionally normal after reinjection.

Although variation between vessels existed within individual animals in the present study, ANOVA revealed that radiation increased platelet recruitment ($P=0.002$). A dose-response effect of irradiation on recruitment at 1 month was seen ($0\text{Gy}<15\text{Gy}<30\text{Gy}$). Importantly, the clinically relevant dose ($15\text{Gy}$) was associated with a doubling of platelet recruitment at this time point compared with controls. The level of recruitment in the control group was $2.7\pm 1.5$, similar to normal nonirradiated nonballoon-injured arteries ($2.9\pm 0.8$).

These data, in concert with the correlative microscopic observations, demonstrate a profound influence of endovascular irradiation in delaying arterial healing and reendothelialization after angioplasty and thereby promote luminal surface thrombogenicity. Although these are interim results in an animal preparation without chronic antiplatelet therapy, they document the potential for thrombosis in this setting. These results suggest that aggressive and prolonged antiplatelet therapy may be helpful in endovascular irradiation for restenosis prevention. This is further substantiated by the appearance of late thrombotic occlusion in recent clinical trials of intracoronary brachytherapy.

Limitations

This study was performed using an animal preparation that mimics some but not all features of coronary angioplasty in the clinical environment, so these findings cannot be used to directly predict responses in that setting.

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