Preserved Endothelium-Dependent Vasodilation in Coronary Segments Previously Treated With Balloon Angioplasty and Intracoronary Irradiation

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Background—Abnormal endothelium-dependent coronary vasomotion has been reported after balloon angioplasty (BA), as well as after intracoronary radiation. However, the long-term effect on coronary vasomotion is not known. The aim of this study was to evaluate the long-term vasomotion of coronary segments treated with BA and brachytherapy.

Methods and Results—Patients with single de novo lesions treated either with BA followed by intracoronary β-irradiation (according to the Beta Energy Restenosis Trial-1.5) or with BA alone were eligible. Of these groups, those patients in stable condition who returned for 6-month angiographic follow-up formed the study population (n=19, irradiated group and n=11, control group). Endothelium-dependent coronary vasomotion was assessed by selective infusion of serial doses of acetylcholine (ACh) proximally to the treated area. Mean luminal diameter was calculated by quantitative coronary angiography both in the treated area and in distal segments. Endothelial dysfunction was defined as a vasoconstriction after the maximal dose of ACh (10⁻⁶ mol/L). Seventeen irradiated segments (89.5%) demonstrated normal endothelial function. In contrast, 10 distal nonirradiated segments (53%) and 5 control segments (45%) demonstrated endothelium-dependent vasoconstriction (−19±17% and −9.0±5%, respectively). Mean percentage of change in mean luminal diameter after ACh was significantly higher in irradiated segments (P=0.01).

Conclusions—Endothelium-dependent vasomotion of coronary segments treated with BA followed by β-radiation is restored in the majority of stable patients at 6-month follow-up. This functional response appeared to be better than those documented both in the distal segments and in segments treated with BA alone. (Circulation. 1999;100:1623-1629.)

Key Words: balloon ■ angioplasty ■ radioisotopes ■ endothelium ■ acetylcholine

Abnormal endothelium-dependent coronary vasomotion has been reported both immediately after and up to 6 months after coronary balloon angioplasty (BA).1-4 The preservation of endothelial function is of the utmost importance to maintain the delicate balance between inhibition and promotion of vascular growth, vasoconstriction, and vasodilation, as well as antiplatelet and antiinflammatory mechanisms.5 Intracoronary radiation appears to be a promising new technique to prevent restenosis after BA.6-8 Experimental studies have demonstrated an impairment of endothelial function in the short term after high-dose intracoronary γ-irradiation, which was restored at follow-up.9 However, the effect of brachytherapy after balloon-induced injury on vasomotor function in patients remains unknown. The aim of the present study was to assess the long-term effect of intracoronary radiation therapy after successful BA on coronary vasomotion.

Methods

Patient Selection

Two groups of patients were compared: patients with single de novo lesions successfully treated with BA followed by intracoronary β-irradiation (n=23) and patients with single de novo lesions successfully treated only with BA (n=16). Patients receiving radiation were included in the Beta Energy Restenosis Trial (BERT-1.5). Patients in the control group were individuals treated with BA alone and matched for age, sex, and vessel size. Those patients in stable condition who returned for 6-month angiographic follow-up formed the study population (n=19, irradiated group and n=11, control group). BERT-1.5 was a prospective multicenter feasibility study. The isotope selected was pure β-emitting ⁹⁰Sr/⁹⁰Y, and patients were randomized to receive 12, 14, or 16 Gy at 2 mm from the source. The
and placed proximally to the irradiated segment. To avoid wire-induced coronary spasm, the wire was removed. The irradiated segment was identified on the basis of the anatomic landmarks visible on the angiogram performed at the time of the placement of the radiation source. To ensure that the segments were fully bathed by the infusion of acetylcholine chloride (ACh), the tip of the infusion catheter was placed 2 to 3 mm proximal to the proximal border of the irradiated area. To determine the baseline vasomotion, an initial infusion of saline solution for 1 minute through the infusion catheter was performed and a baseline angiogram taken. This was followed by infusion of serial doses of intracoronary ACH, with final estimated intracoronary concentrations of $10^{-3}$ to $10^{-5}$ mol/L, to assess endothelium-dependent coronary vasomotion. The duration of each infusion was 2.5 minutes, followed immediately by angiography. All angiograms were taken with identical views and radiographic characteristics. All infusions were delivered at a rate of 2 mL/min by use of a precision pump injector (Mark V, Medrad, Europe BV). The final blood concentrations of ACh were estimated with the assumption that blood flow in the coronary artery was 80 mL/min. Finally, to evaluate endothelium-independent vasomotion, a nitroglycerin (NTG) bolus (2 mg) was administered through the guiding catheter, after which an angiogram identical to those performed previously was done. Throughout each infusion, the heart rate, systemic arterial pressure, and ECG were monitored continuously. Because ACh causes endothelium-dependent vessel relaxation in experimental models and in humans, a paradoxical vasconstriction after the infusion of this substance is an indicator of endothelial dysfunction.

The study was approved by the Medical Ethics Committee of our institution, and written informed consent was obtained from all patients in accordance with the guidelines established by the Committee for the Protection of Human Subjects.

### Quantitative Coronary Angiography

Quantitative coronary angiography was performed after the infusion of saline solution, at the end of each ACh infusion, and after NTG bolus. Angiograms were performed in the 2 orthogonal projections that best showed the artery of interest, without overlapping of side branches and with less foreshortening. Offline analysis was performed by means of the CAAS II system (Pie Medical BV). Calibration of the system was based on dimensions of the catheters not filled with contrast medium. The intraobserver and interobserver variabilities of this method of analysis have been reported previously.

### Statistical Analysis

Data are presented as mean±SD or proportions. To compare continuous variables, 2-tailed Student’s t test, ANOVA for repeated measurements, and linear regression analysis were performed when appropriate. A value of $P<0.05$ was considered statistically significant.
Results

Baseline Characteristics

Baseline characteristics of both irradiated and control patients are presented in Table 1. Angiographic restenosis (diameter stenosis >50%) was observed within the irradiated area in 3 patients (16%). Fourteen patients (74%) remained asymptomatic, whereas 5 (26%) presented with angina pectoris Canadian Cardiovascular Society (CCS) class 1 (n = 1), 2 (n = 1), or 3 (n = 3). None of the patients in the control group showed angiographic restenosis, and only 2 presented with angina pectoris CCS class 1. No differences were observed between groups regarding age, sex, coronary risk factors, or minimal luminal diameter and diameter stenosis in the diagnostic angiogram performed at the time of the functional study. The left anterior descending coronary artery was assessed more often in the control group.

Coronary Vasomotion Study

No significant changes in mean aortic pressure and heart rate were observed during the ACh infusion in either group. Mean luminal diameters after infusion of each substance in irradiated and distal nonirradiated segments and in the control group are presented in Table 2. Seventeen irradiated segments (89.5%) demonstrated normal endothelium-dependent coronary vasomotion (16 segments with a vasodilatory response [5.0 ± 3% of change in mean luminal diameter after ACh] and 1 with no change in mean luminal diameter [−0.1% of change after ACh infusion]). On the other hand, endothelial dysfunction was demonstrated in 2 irradiated segments (10.5%): 1 with angiographic restenosis and angina pectoris CCS class 3 and the other with angina pectoris CCS class 1 without restenosis (−5.2% and −7.8% of vasoconstriction after maximal dose of ACh, respectively). In contrast, 10 (53%) of the distal nonirradiated segments demonstrated endothelial dysfunction (−19.5 ± 17% of vasoconstriction after maximal dose of ACh). No significant de novo stenosis was observed at distal segments. No significant correlation was demonstrated between the degree of stenosis at follow-up and the vasomotor response. Five patients in the control group (45%) showed endothelial dysfunction in the treated area (−9.0 ± 5% of vasoconstriction at ACh 10−6 mol/L). Mean percentages of change in mean luminal diameter after infusion of the different substances between the irradiated and control patients and between irradiated and distal nonirradiated segments are presented in Figures 2 and 3. Mean percentage change in diameter at follow-up ACh was 3.8 ± 7.1% in the irradiated segments compared with −3.2 ± 7% and −6.6 ± 10% in the control group and in the distal nonirradiated segments, respectively (P = 0.01). No significant differences in percentage of change in mean luminal diameter either in irradiated or in distal segments were observed between the 3 coronary vessels after either ACh or NTG. Examples of coronary segments with vasodilation of the irradiated area and vasoconstriction of the distal nonirradiated segment after ACh infusion are depicted in Figures 4 and 5. All of the segments experienced vasodilation after NTG, which is indicative of normal smooth muscle vasomotion (Figures 2 and 3).

Radiation Dose Calculation

Mean prescribed radiation dose was 14 ± 1.9 Gy at 2 mm to the source. However, when dose-volume histograms were applied, the calculated minimal dose received by 90% of the

% change in mean luminal diameter

Figure 2. Percentage of change in mean luminal diameter after infusion of each substance in irradiated segments and in control group. Irradiated segments showed, on average, an increase in mean luminal diameter after infusion of ACh, which is indicative of normal endothelial function, whereas control group demonstrated on average vasoconstriction, which is indicative of endothelial dysfunction. Endothelium-independent coronary vasomotion was preserved in both groups. BL indicates baseline.
luminal surface was 8.2±3.8 Gy, whereas $D_{90\text{ lum}}$ was 5.2±1.9 Gy. Only 6 patients (31.5%) received on average >10 Gy at luminal surface, and only 2 patients (10.5%) received on average >8 Gy at the adventitial layer. No significant correlation was found between endothelium-dependent coronary vasomotion and the calculated $D_{v90\text{ lum}}$ ($r=0.03; P=NS$). Similarly, no significant correlation was observed between the coronary vasomotor response to NTG and $D_{v90\text{ Adv}}$ ($r=0.03; P=NS$).

**Discussion**

This study demonstrates for the first time that the endothelium-dependent vasomotor function of coronary segments treated with BA followed by $\beta$-radiation is restored in the majority of stable patients at 6-month follow-up. This functional response observed in irradiated segments appeared to be better than that documented both in distal nonirradiated segments and in segments treated only with BA.

An impairment of endothelial function has been reported at up to 3 to 6 months after BA. An impairment of endothelial function has been reported at up to 3 to 6 months after BA. It has been demonstrated that soon after balloon-induced injury, there is a release of von Willebrand factor and endothelin as markers of endothelial injury. Experimental studies have demonstrated that the endothelium regenerates at follow-up. However, the endothelium appeared to still be dysfunctional, which may cause the release of endothelium-dependent contracting factors and the alteration of endothelial muscarinic receptors. Endothelial dysfunction in distal nontreated segments is a common finding in atherosclerotic coronary arteries after percutaneous interventions. An alteration of autoregulation due to chronic hypoperfusion may be implicated in the distal abnormal responsiveness to ACh. Furthermore, the presence of coronary risk factors may have a deleterious effect on distal coronary vasomotion.

In contrast, most of the irradiated segments exhibited normal endothelium-dependent vasomotion, and all of them presented a normal response to NTG. Wiedermann et al demonstrated restoration of endothelial function after high-dose (20 Gy) $\gamma$-radiation in a non–balloon-injured animal model. However, a diffuse fibrosis of the smooth muscle layer, probably responsible for the loss of response to NTG, was detected on histological analysis. Our findings confirmed these experimental observations in terms of endothelium-dependent coronary vasomotion. The lack of paradoxical vasoconstriction may be explained by an alteration of the muscular media, which may demonstrate an impairment in response to endothelium-dependent vasoregulatory signals. However, vasodilation rather than lack of constriction was the vasomotor response demonstrated in all but 1 of the irradiated segments with normal endothelial function. The vasomotor response to NTG remained unaltered and comparable between groups, which suggests an absence of radiation-induced impairment of the medial layer. In an experimental model, endothelial cells as well as vascular smooth muscle cells were inhibited in a dose-dependent manner. However, at a moderate range of $\beta$-particle delivery (0.4 to 6 Gy), but not at a high dose (10 Gy), endothelial cells appeared to be more radioresistant than vascular smooth muscle cells. The relatively low dose of radiation received by the treated segments may account for this normal functional behavior. In fact, none

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**Figure 3.** Percentage of change in mean luminal diameter after infusion of each substance in irradiated and in distal nonirradiated segments. Irradiated segments showed, on average, an increase in mean luminal diameter after infusion of ACh, which is indicative of normal endothelial function, whereas distal nonirradiated segments demonstrated, on average, vasoconstriction, which is indicative of endothelial dysfunction. Endothelium-independent coronary vasomotion was preserved in both groups. BL indicates baseline.

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**Figure 4.** Coronary angiogram showing mild vasodilation in irradiated segment at 6-month follow-up.
of the patients actually received on average >10 Gy at the level of the adventitia, as assessed by dose-volume histograms.

On the other hand, an experimental model of porcine coronary arteries subjected to balloon overstretch injury and either placebo or radiation with 18 Gy demonstrated that expression of enzyme-inducible nitric oxide synthase (iNOS), responsible for NO production, was enhanced, whereas expression of the cytokine transforming growth factor-β (TGF-β) was suppressed in the irradiated group. iNOS is potentially responsible for inhibition of neointimal hyperplasia and stimulation of reendothelialization, whereas TGF-β would enhance intimal hyperplasia and fibrosis by negatively modulating the expression of iNOS. Moreover, it has been demonstrated in experimental models that radiation causes dose- and time-dependent impairment of endothelium-dependent relaxation and that low-dose radiation would induce an anti-inflammatory reaction through specific dose-dependent modulation of the NO pathway. It remains to be seen whether this chain reaction after radiation would result in a late reduction in the restenosis rate. However, restoration of endothelial function may play an important role in this regard.

Study Limitations
Because the use of ACh in unstable patients is not exempt of risk of coronary occlusion, only stable patients were evaluated.

This study assessed patients receiving β-radiation by means of a noncentering device. The actual dose received by the treated segment was rather low; thus, the effect of a higher dose or of different devices that allow a more homogeneous dose distribution remains to be evaluated.

We assessed the vasomotion of the 3 coronary arteries in the irradiated group, which have a potentially different degree of vasoreactivity to ACh. However, the 3 arteries demonstrated comparable vasomotor responses to ACh and NTG both at the irradiated and the distal segments, which overcomes this potential limitation.

We assumed that coronary vasomotion immediately after treatment is markedly impaired, as demonstrated in experimental models and in humans. Taking into account the risk of coronary occlusion in such situation, it was considered unethical to determine endothelium-dependent vasomotion immediately after the coronary intervention. Thus, the degree of recovery of coronary vasomotion could not be evaluated.

We also assumed that the IVUS and delivery catheters were lying in the same position in the treated coronary
segment. The size of the IVUS catheter is smaller (2.9F, \(\approx 1\) mm) than the brachytherapy device (5F), which is thus to some extent more centered in the lumen. Although the catheters should be on the shortest 3D path in the lumen, coronary arteries have a complex curved geometry in space and can be partially deformed by the catheters. Thus, catheters with different rigidity may occupy different positions. The development of new systems that incorporate the IVUS imaging element on the delivery catheter might resolve this drawback.

During irradiation, the position of the delivery catheter inside the lumen is not fixed and may vary during the cardiac cycle because of ventricular contractions, which may lead to some degree of inhomogeneity not assumed by data derived from the static end-diastolic IVUS images.

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