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Radiation Therapy Impairs Endothelium-Dependent Vasodilation in Humans

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OBJECTIVES The objective of this study was to test the hypothesis that external-beam radiation induces a chronic impairment of endothelium-dependent vasodilation.

BACKGROUND Radiation therapy is used commonly in the treatment of cancer and is associated with an increased incidence of adverse vascular events related to the field of radiation, including stroke and myocardial infarction. As endothelial injury is central to the pathogenesis of vascular diseases, we hypothesized that radiotherapy induces arterial endothelial dysfunction.

METHODS Sixteen women with unilateral breast cancer who underwent standard external-beam radiation therapy to the breast and axilla >3 years before enrollment and ten healthy women were studied. Vascular ultrasonography was used to image both the artery exposed to radiation and the contralateral artery. Flow-mediated, endothelium-dependent vasodilation and endothelium-independent vasodilation to nitroglycerin of both axillary arteries were measured.

RESULTS Endothelium-dependent vasodilation was significantly impaired in the irradiated axillary arteries compared with the contralateral, nonirradiated arteries ($-0.4 \pm 0.4\%$ vs. $3.2 \pm 0.8\%$, $p < 0.001$) and also compared with control subjects' arteries ($-0.4 \pm 0.4\%$ vs. $2.5 \pm 0.6\%$, $p < 0.001$). In contrast, endothelium-independent vasodilation was greater in the arteries that received radiation compared with the contralateral arteries ($3.8 \pm 0.5\%$ vs. $2.0 \pm 0.4\%$, $p < 0.05$) and also compared with control arteries ($3.8 \pm 0.5\%$ vs. $2.5 \pm 0.4\%$, $p < 0.05$).

CONCLUSIONS External beam radiation therapy impairs endothelium-dependent vasodilation of conduit arteries, implicating a decrease in the bioavailability of nitric oxide. These abnormalities may contribute to the development of arterial occlusive disease and associated clinical events. (J Am Coll Cardiol 2001;37:761–5) © 2001 by the American College of Cardiology

External-beam radiation therapy is commonly employed as a primary and adjuvant therapeutic modality in patients with neoplasms. Its mechanism of action is thought to involve direct DNA damage, which causes cell death (1). Despite improving technology and dose delivery, normal "bystander" tissues, including the vasculature, are often affected. Indeed, radiation therapy increases the incidence of arterial occlusive disease years after therapy. The arterial lesions become manifest in the region of previous radiation and can cause clinical events in the cerebrovascular (2–5), coronary (6–8) and peripheral circulations (9).

The mechanism whereby radiation therapy causes arterial stenoses after radiation exposure is unclear, but important consideration must be given to impairment of endothelial-cell function. Decreased bioavailability of endothelium-derived nitric oxide may contribute to vascular injury by facilitating platelet aggregation, increasing the adherence of leukocytes to the endothelial surface and by promoting growth of vascular smooth cells (10). Abnormalities in endothelium-dependent vasodilation and, by extension, endothelium-derived nitric oxide have been demonstrated to accompany risk factors for atherosclerosis (11–15) and may also account for the predisposition to vascular disease in arteries exposed to radiation. Abnormal endothelium-dependent vasodilation has been demonstrated in animal models and humans in vitro and in animals in vivo (16–19) acutely after radiation exposure. There has been no investigation to determine if the effect of radiation therapy on the endothelium persists chronically after radiation exposure, thus providing a substrate for arterial disease.

Accordingly, the purpose of this study was to test the hypothesis that endothelium-dependent vasodilation is impaired in vivo in human arteries exposed to radiation. We used high-resolution ultrasound to measure endothelium-dependent and endothelium-independent vasodilation in the irradiated and contralateral nonirradiated axillary arteries of women who underwent unilateral radiation therapy for breast cancer.

METHODS

Patient selection and recruitment. Sixteen postmenopausal women who had received unilateral adjunctive axillary radiotherapy for breast cancer >3 years earlier were eligible for enrollment. Subjects were recruited from the clinic of one of the investigators (J.R.H.). Ten healthy,

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Abbreviations and Acronyms
BP = blood pressure
LDL = low density lipoprotein
MI = myocardial infarction

postmenopausal, age-matched women were also recruited via advertisement and invited to participate in the study. Exclusion criteria included diabetes mellitus, cigarette smoking, total and low density lipoprotein (LDL) cholesterol >75th percentile for age, untreated hypertension, clinical manifestations of atherosclerosis, evidence of recurrent or second malignancy, severe lymphedema or other major medical problem. All subjects underwent pre-enrollment history and physical examination. Each subject provided written, informed consent. This study was approved by the Human Research Committee of Brigham and Women's Hospital.

Vascular reactivity studies. Subjects were studied in a quiet, temperature-controlled, dimly lit room after resting supine for 5 min. High-resolution ultrasonography of the axillary artery was performed using a Toshiba 270 SSA ultrasound machine and 7.5 MHz linear array probe. The axillary artery was imaged longitudinally just distal to the axillary-subclavian junction, with the arm abducted 90° from the body. The transducer position was adjusted to obtain optimal images of the near and far walls of the intima. Baseline images were simultaneously recorded on super VHS videotape. The video output and electrocardiographic signal of the ultrasound machine were connected to a computer equipped with a Data Translation frame-grabber videocard, (Dataviz, Trumbull, Connecticut). The 'R' wave on the electrocardiogram was used as a trigger to acquire frames at end-diastole. After baseline image acquisition, a mid-arm sphygmomanometric cuff was inflated to suprasystolic pressure for 5 min. Upon cuff release, reactive hyperemia causes flow to increase through the conduit artery subserving the arm, in this case the axillary artery. Flow-induced, endothelium-dependent vasodilation of the axillary artery was determined via imaging at 1 min after cuff deflation. Flow-mediated vasodilation at this time point is largely nitric oxide mediated and endothelium dependent and can be inhibited by administration of the nitric oxide synthase antagonist, N^G-monomethyl-L-arginine (20). Ten minutes after cuff release, the axillary artery was imaged again to re-establish baseline conditions. Then, to determine endothelium-independent vasodilation, subjects received 0.4 mg of nitroglycerin, sublingually. The axillary artery was imaged 3 min later. Axillary artery blood flow velocity was determined via time-velocity integral measurement and is reported as cm/s. At least 15 min after the administration of nitroglycerin and after re-establishment of pre-nitroglycerin heart rate and blood pressure (BP), the procedure was repeated in the contralateral axillary artery. Nitroglycerin was not administered if the systolic BP was below 110 mm Hg.

Image analysis. Acquisition and analysis of the stored images were performed using software designed for this purpose (Information Integrity, Inc., Cambridge, Massachusetts). At least four end-diastolic images for each condition (baseline, reactive hyperemia, repeat baseline, nitroglycerin) were selected for analysis. Analysis was performed by one investigator (J.A.B.) who was blinded to subject name, date and presence or absence of radiation exposure. The near and far walls were determined by derivative-based edge detection after identification of the region of the anterior and posterior walls by the investigator. The maximum diameter of the vessel was then determined. Arterial diameter was measured from the vessel-lumen interface on the posterior wall to the vessel-lumen interface of the anterior wall.

Statistical analysis. Descriptive statistics are reported as mean ± standard deviation. Vasodilation is reported as mean ± standard error of the mean. Endothelium-dependent and endothelium-independent vasodilation in the axillary artery exposed to radiation therapy were first compared using repeated measures analysis of variance. Post-hoc, endothelium-dependent and endothelium-independent vasodilation in the axillary artery exposed to radiation therapy were compared with that of the contralateral nonirradiated artery using a paired Student *t* test. Comparisons of endothelium-dependent and independent vasodilation of the irradiated arteries of patients to axillary arteries of healthy control subjects were made using an unpaired *t* test. Regression analysis was used to examine the relationship between endothelium-dependent vasodilation and selected subject characteristics. Statistical significance was accepted at the $p < 0.05$ (95% confidence interval) level.

RESULTS

Baseline characteristics. Twenty-six postmenopausal women, including 16 subjects with prior radiation therapy and 10 healthy control subjects, were enrolled. By group, the participants were aged 58 ± 10 and 58 ± 4 years, had a mean BP of 90 ± 13 and 91 ± 9 mm Hg, had a total cholesterol of 203 ± 21 and 212 ± 20 mg/dl, and LDL of 121 ± 17 and 116 ± 22 mg/dl for the radiation-therapy subjects and healthy control subjects, respectively. There were no significant between group differences (all p = NS). The average length of time between the last radiation therapy and enrollment was 12 ± 6 years. Nine patients had had chemotherapy. One patient was taking tamoxifen. In the radiation therapy group, three subjects had a history of hypertension; two were taking beta-adrenergic blocking agents, and one patient was taking an angiotensin-converting enzyme inhibitor. They were asked to hold these medications on the study day.

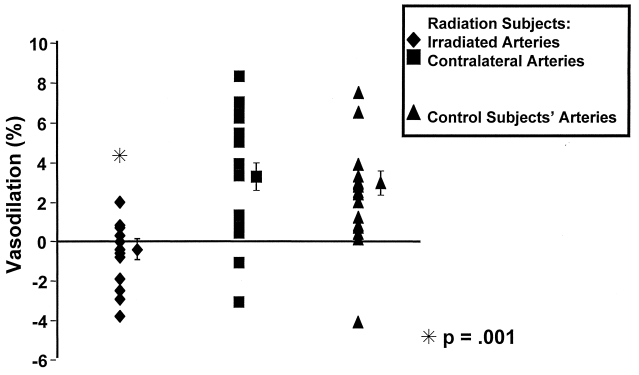

Figure 1. Effect of radiation therapy on flow-mediated vasodilation. The individual and mean percent increase in axillary artery size 1 min after cuff release compared with baseline is illustrated. Error bars represent standard error. Flow-mediated vasodilation was significantly impaired in irradiated arteries compared with nonirradiated arteries and arteries in control subjects ($p < 0.001$).

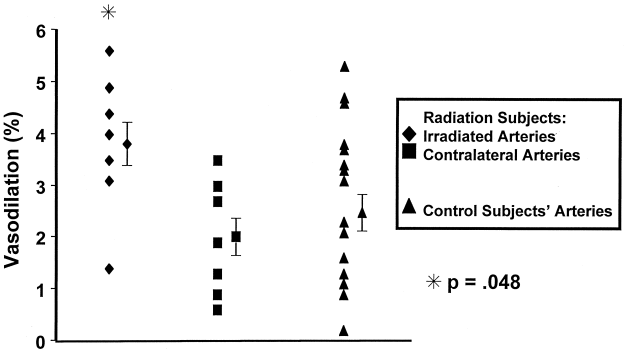

Figure 2. Effect of radiation therapy on nitroglycerin-mediated vasodilation. The individual and mean percent increase in axillary artery size after nitroglycerin administration is shown. Error bars represent standard error. Nitroglycerin-mediated vasodilation was significantly greater in irradiated arteries compared with nonirradiated arteries and arteries in control subjects ($p < 0.05$).

Effect of radiation therapy on endothelium-dependent vasodilation. The baseline diameters of the axillary arteries in the subjects who received radiation therapy were 5.3 ± 0.9 mm and 5.7 ± 0.7 mm for the irradiated and nonirradiated contralateral arteries, respectively ($p = 0.06$). Reactive hyperemia augmented axillary artery flow velocity 240% (from 9.8 cm/s to 23.8 cm/s) in the irradiated arteries and 260% (from 8.2 cm/s to 22.8 cm/s) in the nonirradiated, contralateral arteries. The increase in blood flow velocity was not significantly different in the irradiated and nonirradiated arteries ($p = 0.62$). Flow-mediated, endothelium-dependent vasodilation was lower in the arteries exposed to radiation therapy than it was in the contralateral arteries and in the arteries of the healthy, control subjects ($p = 0.001$ by analysis of variance) (Fig. 1). In a direct comparison between the irradiated arteries and the contralateral arteries, the irradiated arteries did not vasodilate, while the contralateral arteries did (−0.4 ± 0.4% vs. 3.2 ± 0.8%, respectively; $p < 0.001$).

The baseline diameter of the axillary arteries in the healthy, control subjects was 5.7 ± 0.7 mm, and this was not significantly different from the unexposed (i.e., nonirradiated) axillary arteries of the patients who received radiation therapy ($p = 0.75$). Flow-mediated vasodilation of the axillary arteries of control subjects averaged 2.5 ± 0.6% and did not differ between arms. Flow-mediated vasodilation was significantly greater in the axillary arteries of control subjects than it was in the arteries of patients exposed to radiation therapy (2.5 ± 0.6% vs. −0.4 ± 0.4%, respectively; $p < 0.001$) (Fig. 1). Flow-mediated vasodilation did not differ significantly between the nonirradiated axillary arteries of the subjects who received radiation therapy and the axillary arteries of control subjects (3.2 ± 0.8% vs. 2.5 ± 0.6%, $p = 0.51$).

Effect of radiation therapy on exposed artery endothelium-independent vasodilation. Seven of the patients who had previously received radiation therapy were given nitroglycerin to determine endothelium-independent vasodilation. Of those who did not receive nitroglycerin, eight subjects had systolic BP below 110 mm Hg, and one subject refused nitroglycerin. Endothelium-independent vasodilation was significantly greater in the irradiated arteries compared with the nonirradiated arteries and the arteries of the control subjects ($p = 0.048$, by analysis of variance). In a direct comparison, endothelium-independent vasodilation was significantly greater in the irradiated arteries compared with the nonirradiated arteries (3.8 ± 0.5% vs. 2.0 ± 0.4%, $p < 0.05$) (Fig. 2). The arteries exposed to radiation therapy also had a greater response to nitroglycerin than those of the control subjects (3.8 ± 0.5% vs. 2.5 ± 0.4%, respectively; $p < 0.05$).

Multivariate analysis. Subject age, total and LDL cholesterol, BP, previous administration of chemotherapy and length of time since radiotherapy administration did not affect the flow-mediated and nitroglycerin-mediated vasodilation of irradiated and nonirradiated arteries (all p = NS by regression analysis). There was no significant interaction between the vasodilation and any of the above factors (p = NS). There was no significant difference in flow-mediated vasodilation or nitroglycerin-mediated vasodilation between arms of the control subjects ($p > 0.2$ for both comparisons) (Fig. 3).

DISCUSSION

The novel finding of these experiments is that external-beam radiation therapy causes chronic impairment of endothelium-dependent vasodilation in humans in vivo, specific to arteries that received radiation. This observation suggests that endothelial dysfunction may contribute to the increased incidence of arterial occlusive disease that occurs in patients who received radiation therapy.

Radiation modifies endothelial-cell function and reduces its survival. Radiation causes the endothelial release of a neutrophil chemoattractant, up-regulates endothelial expression of cellular adhesion and diapedesis molecules and increases endothelial cell production of platelet derived

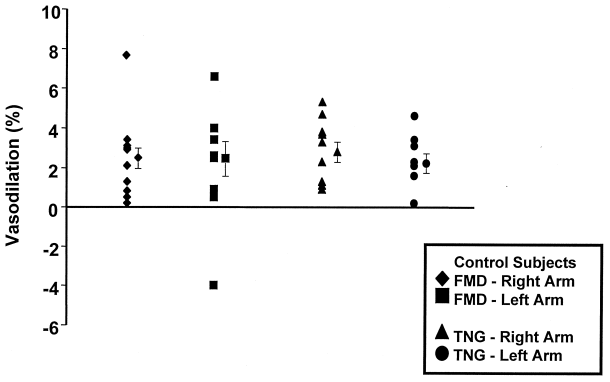

Figure 3. Inter-arm variation between healthy control subjects. The individual and mean flow-mediated and nitroglycerin-mediated vasodilation in the right and left arms of the control subjects. Error bars represent standard error. There is no significant difference between arms for either flow-mediated or nitroglycerin-mediated vasodilation.

growth factor (21–23). Irradiation of muscular (conduit) arteries results in endothelial cell death, leaving the luminal surface sparsely populated at six weeks. Irregular appearing cells begin to repopulate the luminal surface 6 to 10 weeks later (24).

Radiation and endothelium-derived nitric oxide. Studies in animals and humans in vitro and animals in vivo suggest that bioavailability of endothelium-derived nitric oxide is diminished shortly after radiation exposure (16–19). Endothelium-dependent vasorelaxation to acetylcholine and calcium ionophore in irradiated rabbit-ear arteries in vitro is reduced, whereas nitroprusside-induced vasorelaxation is preserved (18). In rats receiving prostate radiation, in vivo erectile function and the number of nitric oxide synthase-containing nerve fibers per corpus callosum is decreased (19). In humans, endothelium-dependent vasodilation is impaired in vitro, and tissue endothelial nitric oxide synthase is decreased in carotid arteries four to six weeks after neck irradiation (16). Our in vivo findings of impaired endothelium-dependent vasodilation in irradiated arteries extend these observations. We observed that endothelium-dependent vasodilation was diminished in irradiated arteries years beyond therapy.

There was a trend toward a smaller baseline diameter of the irradiated axillary arteries compared with the contralateral, nonirradiated axillary arteries and the axillary arteries of the healthy, control subjects. The mechanisms whereby radiation therapy may have reduced arterial diameter are not known, but decreased nitric oxide is a plausible possibility. Normal levels of nitric oxide are known to protect against cellular apoptosis (25,26). The smaller baseline size may reflect increased cellular apoptosis within the vessel as a consequence of decreased nitric oxide bioavailability. Endothelium-derived nitric oxide is also a potent vasodilator and important in the regulation of arterial tone. Thus, chronically diminished constitutive nitric oxide production may result in a persistently smaller resting vessel lumen.

Radiation therapy and endothelium-independent vasodilation. Endothelium-independent vasodilation was increased in the irradiated axillary arteries compared with the nonirradiated contralateral arteries and the arteries of control subjects. This finding may reflect the fact that smaller arteries dilate proportionally more than larger arteries when exposed to a vasodilator stimulus (27) or occur as a compensatory response to any nitric oxide donor when endogenous nitric oxide is reduced (28).

Clinical significance. This study demonstrates that exposure to radiation chronically impairs endothelial function. Endothelial dysfunction is a form fruste of atherosclerosis and may account, in part, for stenoses that occur in irradiated arteries, such as the coronary, carotid and subclavian/axillary circulations. Patients who receive mantle or head and neck radiation therapy have an increased rate of myocardial infarction (MI) (6–8) and stroke (2–5). In fact, in women under age 60 who receive left chest irradiation, the risk of MI is doubled (29). For younger patients who survive their cancer, long-term morbidity and mortality is increased by the life-saving radiotherapy. Our study provides evidence that endothelial dysfunction may be a target for therapy to prevent these adverse outcomes.

Conclusions. The results of this study enable us to conclude that radiation therapy causes impairment of endothelium-dependent vasodilation in exposed arteries. The findings implicate reduced bioavailability of endothelium-derived nitric oxide as an important mechanism for the subsequent development of arterial occlusions and vascular events in patients receiving radiation therapy.

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