Gamma Irradiation Inhibits Neointimal Hyperplasia in Rats After Arterial Injury

Steven Shimotakahara, MD; Marc R. Mayberg, MD

**Background and Purpose** Restenosis complicates a significant proportion of endovascular and open vascular procedures such as carotid endarterectomy. In contrast to the primary atheroma, restenosis is characterized by intimal hyperplasia of vascular smooth muscle cells. We hypothesized that gamma radiation would reduce restenosis by limiting intimal hyperplasia after arterial injury.

**Methods** To demonstrate the effect of gamma radiation on smooth muscle hyperplasia in vivo, a standardized bilateral carotid balloon catheter arterial injury was produced in 37 rats. A single dose of 750, 1500, or 2250 cGy (1cGy=1 rad) gamma radiation was delivered to the right carotid artery at either 1 or 2 days after injury; the shielded contralateral carotid artery served as matched control. At 21 days after injury, vessels were perfusion-fixed in situ, and cross-sectional area of neointima was determined from axial sections using image analysis.

**Results** Marked reductions in neointimal cross-sectional area were demonstrated in vessels subjected to 1500- and 2250-cGy radiation at both 1 and 2 days after injury. A less prominent effect was noted for 750 cGy, reaching statistical significance only at 2 days after injury. By two-way ANOVA, radiation dose (P=.0002), timing of radiation delivery (P=.003), and an interaction between timing and dose (P=.0278) were significantly associated with reduction in neointimal cross-sectional area. At 1500 cGy, delivery of radiation 1 day after injury inhibited neointimal hyperplasia more prominently than the same dose 2 days after injury; a dose-response relation was evident at 1 day.

**Conclusions** Radiation may be an important adjunctive therapy for reducing the incidence of restenosis after angioplasty or endarterectomy. (Stroke. 1994;25:424-428.)

**Key Words** • angioplasty • carotid endarterectomy • muscle, smooth • radiation • rats

**Materials and Methods** Thirty-seven male Sprague-Dawley rats (Simonsen, Gilroy, Calif) weighing 350 to 400 g were anesthetized with intraperitoneal ketamine 100 mg/kg, xylazine 6.25 mg/kg, and atropine 2 mg/kg. All procedures were performed in accordance with guidelines established by the Animal Care Committee at the University of Washington. As previously described, both carotid bifurcations were exposed through a midline cervical incision, the external carotid arteries were cannulated with a 2F...
Arteries irradiated at 2 days after injury (not shown) 750 cGy administered at 1 day after injury (Fig lb). In neointimal hyperplasia compared with controls (Fig lc and Id). Intermediate reductions were observed for 1500 cGy (40% reduction; P<.05), 2250 cGy (73% reduction; P<.01).

There was a significant reduction in neointimal area at 1500 cGy administered at 1 day after injury compared with 2 days (P=.035), with a trend for a similar effect noted for 2250 cGy (P=.149) but not 750 cGy (P=.690). By one-way ANOVA, increasing radiation dose was significantly associated with reduction in neointimal area at 1 day (P=.0024) but not at 2 days (P=.155) after injury. By two-way ANOVA testing, both radiation dose (P=.0002) and timing of radiation delivery (P=.003) were associated with reduction in neointimal cross-sectional area; moreover, there was an interaction between these two variables in their effect (P=.028).

Discussion
Smooth muscle cell hyperplasia following arterial vessel wall injury has been studied extensively using the balloon catheter model employed in this experiment.27 In this model, SMC proliferation begins in the media, followed by migration of cells through the internal elastic lamina into the intima and subsequent intimal SMC proliferation to generate a neointimal layer. In this process, normally nonregenerating vascular SMCs are stimulated by injury and a variety of mitogenic factors to become actively proliferating tissue. Majesky et al28 showed that after balloon catheter arterial injury, SMC ornithine decarboxylase activity (indicating SMC entry into prereplicative G1 phase) peaked at 6 hours with a rapid falloff by 9 hours, whereas the [3H]thymidine index (S phase) was maximal at 33 hours with a rapid decline by 48 hours. Although thymidine uptake by SMCs may persist in the neointima adjacent to the luminal surface for up to 12 weeks, there is no apparent increase in SMC accumulation after 2 weeks in this model.27 Similarly, the antiproliferative effect of intravenous heparin in the balloon injury model was most pronounced in the first 18 hours after injury.28 These findings suggest that SMCs rapidly and synchronously enter the replicative cell cycle after the injury event. This cohort or clone of cells is presumably the progenitor of continued proliferation, which eventually results in neointimal hyperplasia.29 Although the proliferative index of SMCs following radiation was not measured in this experiment, radiation likely inhibited SMC hyper-
Photomicrographs of elastin-stained rat carotid artery in cross section at 21 days after balloon catheter arterial injury. Nonradiated control arteries (a) showed typical intimal smooth muscle cell hyperplasia medial to internal elastic laminae with luminal narrowing. Arteries treated with external gamma radiation administered at 1 day after injury demonstrated progressive inhibition of neointimal hyperplasia with increasing dose: 750 cGy (b), 1500 cGy (c), or 2250 cGy (d). Similar less prominent inhibition of intimal hyperplasia was noted in histological sections from animals radiated at 2 days after injury.

plasia by either killing progenitor cells or limiting their reproductive capacity during early cell division, thus reducing the number of clonal populations. In addition, we noted that the effect of radiation on SMC hyperplasia was more pronounced at 1 day after injury than at 2 days, suggesting that SMCs enter into the proliferative phase as a synchronous cohort of cells. Other models of radiation injury have shown that dividing cells are most susceptible to the effects of radiation during metaphase, when DNA and chromosomes undergo rearrangement injuries.30 If SMCs enter the proliferative phase as a clone rather than sequentially, further time response studies should identify the exact window of maximal radiosensitivity and determine whether radiation inhibits intimal hyperplasia for periods longer than 21 days. This would not only lend insight into the events leading to SMC proliferation but may be valuable in defining the least amount of radiation that would be effective in suppressing the SMC response. Clinical applicability would depend in large part on such information if radiation were used to treat neointimal proliferation after vascular procedures.

Dose-response relations have been delineated for most actively dividing normal tissues with in vivo assays of clonogenic populations.31-34 Most in vivo assays of radiosensitivity require relatively high single-dose radiation (800 to 1600 cGy) to produce sufficient biologic damage, and the effect of smaller doses of radiation must be estimated from fractionated radiation schedules. Confluent nondividing mouse mesentery SMCs in vitro demonstrate very slow turnover rates after exposure to 2000- and 4500-cGy gamma irradiation.35 In contrast, prolifer-
Single-fraction radiation doses up to 1500 cGy are most effective within the first 48 hours after injury. Angioplasty coils to produce neointimal hyperplasia in vivo. Herbaux et al. used implanted metallic stents to produce neointimal hyperplasia in vivo. Rat aortic SMCs in exponentially growing cell cultures were moderately radiosensitive, with $D_0$ values of 120 to 160 cGy and extrapolation distances of 2 to 10.5,6,7 Few studies have examined the effects of gamma radiation on SMC hyperplasia in vivo. Herbaux et al. found a qualitative increase in intimal hyperplasia in the region of arterial anastomosis in rats when radiation was delivered in weekly fractions to total doses of 2370 to 3080 cGy. Based on our data, it is likely that radiation in this study was administered beyond the period of maximal efficacy. Schwartz et al. used implanted metallic angioplasty coils to produce neointimal hyperplasia in pig coronary arteries. A small increase in neointimal cross-sectional area was noted in groups receiving 400- cGy external-beam gamma radiation at 1 day after angioplasty and divided 400 cGy fractions at 1 day and 4 days after injury. Our data suggest that the minimal effective single dose to inhibit SMC proliferation is more than 750 cGy and that fractionated doses would be most effective within the first 48 hours after injury. Single-fraction radiation doses up to 1500 cGy are generally well tolerated in humans,8,9 so that radiation in the effective range for preventing intimal hyperplasia for this experiment (1500 cGy) could be clinically applied. In addition, an equivalent radiobiologic effect with less injury to normal tissues can be achieved using multifraction administration at lower radiation doses.

In the current study, single-fraction external-beam gamma radiation at doses between 750 cGy and 2250 cGy reduced neointimal hyperplasia in rat carotid arteries following balloon arterial injury. Radiation was more effective when administered at 1 day after injury compared with 2 days. The mechanism by which radiation inhibited neointimal hyperplasia in this model is indeterminate but likely involved damage to early progenitor cells during cell division, thus reducing the number of clonal populations. It is less likely that radiation reduced SMC hyperplasia by inhibiting migration from the media to the intima, since migration of SMCs in vitro was not inhibited by external radiation.37 Nevertheless, SMCs may respond differently in vivo because of local effects of chemotactic factors.38,39 We believe that gamma irradiation causes a reduction in neointimal proliferation by interfering with DNA synthesis and repair in actively dividing SMCs.

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**References**

Angioplasty has been used widely during the past decade to treat patients with arterial stenosis, especially in coronary arteries. Angioplasty is remarkably effective in dilating stenotic lesions, but restenosis is very common. Many approaches have been examined to prevent restenosis, but no drug or procedure has been consistently effective.

The article by Shimotakahara and Mayberg is a novel approach to restenosis, and it is a potentially important first step to treatment. Radiation to inhibit the hyperplastic response of vascular muscle after arterial injury is a clever idea. One must be cautious in extrapolation of the findings because the dose of radiation is large, sophisticated approaches will be needed to confirm the supposition that radiation inhibits neointimal proliferation, and the mechanism of the effect is obscure. Nevertheless, this new idea for treatment of arterial restenosis certainly is attractive.

Donald D. Heistad, MD, Guest Editor
Department of Internal Medicine and Pharmacology
University of Iowa College of Medicine
Iowa City

Editorial Comment

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S Shimotakahara and M R Mayberg

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