

● *Biology Original Contribution*

THE RADIOBIOLOGY OF INTRAVASCULAR IRRADIATION

DAVID J. BRENNER, D.Sc., RICHARD C. MILLER, Ph.D. AND ERIC J. HALL, D.Sc.

Center for Radiological Research, College of Physicians and Surgeons of Columbia University, New York, NY

Purpose: There is increasing interest in the use of vascular irradiation, from an internally introduced radioactive source to control restenosis after balloon angioplasty. Both animal experiments and early clinical studies appear to show promising results in this regard. We consider various mechanistic interpretations of the experimental and clinical observations that doses of 12–20 Gy appear to be efficacious in preventing restenosis. We develop and investigate simple models, both experimental and theoretical, of the kinetics of radiation-induced smooth muscle cell (SMC) inactivation and regrowth, as a first step toward optimizing the design of clinical vascular irradiation.

Methods and Materials: Using *in vitro* models of human SMCs, we investigate the relative radiosensitivity of SMCs compared with endothelial cells and measure the dose-dependent ability of SMCs to repopulate a denuded region in a confluent layer of cells. We then use quantitative information on the number, radiation sensitivity, and growth rate of the potential arterial target cells to model the time course of the SMC population after irradiation.

Results and Conclusion: Doses >20 Gy, which would be required to completely eliminate the SMC population which has the potential to cause restenosis, are too large to be practical because of the unacceptable risk of late complications. However, doses that can be practically given in vascular irradiation (<20 Gy) will certainly delay restenosis by 1–3 years, with larger doses producing longer delays. Whether such doses can avert restenosis permanently is unclear, as permanent prevention at realistic doses depends critically on the assumption that those SMCs which survive irradiation have a significantly limited capacity for proliferation. With regard to current animal model experiments, routine follow-up of <1 year, which is standard practice, is probably too short to address some of the key mechanistic question in intravascular radiation therapy. Copyright © 1996 Elsevier Science Inc.

Restenosis, Radiation, Percutaneous transluminal coronary angioplasty (PTCA).

INTRODUCTION

One of the major drawbacks to percutaneous transluminal coronary angioplasty (PTCA) is the 40–60% incidence of restenosis (13). The basic mechanism for restenosis (3, 4) is believed to be induced proliferation and migration from the media of smooth muscle cells (SMC), possibly in conjunction with a failure of the internal elastic lamina to enlarge correspondingly (10). There is much interest, therefore, in the use of vascular irradiation, delivered through an internally introduced radioactive source, to control restenosis after PTCA.

Both animal studies (12, 14, 16, 20–25) and early clinical studies (11, 26, 27) of vascular irradiation after PTCA appear to indicate that the technique has considerable promise in controlling restenosis. In this communication, we consider various mechanistic interpretations of these experimental and clinical observations: specifically that radiation doses of 12–20 Gy appear to be efficacious in preventing restenosis.

Three possible interpretations of these observations are:

1. If SMCs are inherently significantly more radiosensitive than endothelial cells, then the radiation might inactivate all of the SMCs and allow the endothelial cells to repopulate and reline the artery. However, there is little experimental evidence suggesting large differences in the radiosensitivity of different types of normal human cells (7).
2. The population size of potentially clonogenic SMCs in the vessel might be such that depopulation by a relatively small factor (rather than complete depopulation) would eliminate the possibility that the surviving SMCs could proliferate sufficiently to cause restenosis. This is plausible because these SMCs are not immortal, and there is convincing evidence (9) that normal cells have a finite proliferative capacity, both *in vivo* and *in vitro*.
3. A third interpretation is that the experimental observations to date reflect a growth-delay situation, in

which the inhibition of restenosis is simply delayed for the period of time necessary for the population of SMCs to regenerate, after which restenosis can occur as before. If this were the case, vascular irradiation would delay restenosis rather than prevent it.

In this report, we develop and investigate simple models, both experimental and theoretical, of the kinetics of radiation-induced SMC inactivation and regrowth as a first step toward optimizing the design of clinical vascular irradiation.

METHODS AND MATERIALS

In vitro studies

We have investigated the issue of the relative radiosensitivity of endothelial and SMC cells, each of human origin. Figure 1 shows *in vitro* cell survival results for aortic SMCs and endothelial cells, both of human origin. The two cell types are similar in their response to radiation, and their radiosensitivities are unremarkable in that they fall within the range of those of most mammalian cells (7). For example, after a dose of 8 Gy, the fraction of cells surviving is about 10^{-2} , i.e., approximately 1 in 100 cells remains clonogenic.

We next consider the significance of the fact that the SMCs are normal, nonmalignant cells, and are therefore capable of only a limited number of divisions before senescence occurs. That this is a central issue can be seen in the following simple example: Suppose 10^8 new SMCs were needed to produce restenosis, but SMCs were capable of no more than about 10 divisions (a proliferation factor of about 10^3). Then reducing the local SMC population below 10^5 would be adequate to prevent restenosis. However, if the SMCs were capable of 20 divisions (a proliferation of about 10^6), the local SMC population would need to be reduced to $<10^2$ to prevent restenosis. At the extreme, if the SMCs were capable of 30 divisions (a proliferation of about 10^9), the local SMC population would need to be completely eliminated to prevent restenosis.

To investigate this issue, an *in vitro* experiment was set up with short-term cultures of human aortic SMCs. The experiment was designed as a simple model of the kinetic processes occurring when balloon angioplasty causes a tear of several millimeters in the endothelial lining of an artery, allowing SMCs to proliferate through the gap.

Aortic SMCs (derived from a 54-year-old male) were plated into 100-mm petri dishes and allowed to reach confluence and stop dividing. A sterile rubber policeman 3 mm wide was then used to wipe cells clean across a diameter of each dish. Replicate dishes were immediately exposed to graded x-ray doses of 0–20 Gy. Dishes were inspected and photographed daily to record the kinetics of the cells as they repopulated the narrow band that had been denuded of cells.

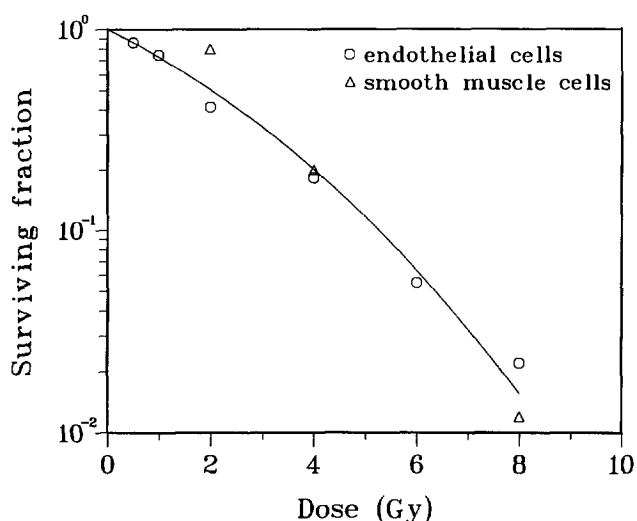


Fig. 1. Survival data for aortic SMCs and for endothelial cells, both of normal human origin, exposed *in vitro* to graded doses of X rays. There is no significant difference in the radiation sensitivity of the two cell types, although comparisons should be tempered by the fact that the two cell lines originated from different individuals. The curve represents a fit of the surviving fraction (S), as a function of radiation dose (D), to the linear-quadratic formula, $S=e^{-\alpha D-\beta D^2}$, where α and β are free parameters.

In this model system, in the absence of radiation, cells invade the denuded band, which was repopulated, and become confluent in about 10 days. Doses of around 10 Gy delay this process, whereas at doses of 15 and 20 Gy, the gap is never bridged by the SMCs, even at long times after irradiation. As discussed below, this is presumably because the surviving cells senesce before sufficient divisions can be achieved. This process is quantitated in Fig. 2.

As we discuss later, it is important to recognize that the quantitative aspects of this *in vitro* experiment are unlikely to be translatable directly to the clinical situation, but the experiment does illustrate qualitatively the consequences of a limited potential for cell division in surviving SMCs.

Modeling studies

Our approach is to use quantitative information on the number, radiation sensitivity, and growth rate of the potential arterial target cells, to model the time course of the SMC population after irradiation.

Let us consider a 20-mm arterial segment, schematized in Fig. 3, with external and internal diameters of 3 and 1.5 mm, respectively. If this is irradiated with an axial source, a reasonable estimate of the density of medial cells implies that the number of cells irradiated in the media is $\sim 15 \times 10^7$ cells. Of course, not all of these cells are proliferating, even under the stimuli of PTCA and/or radiation, and any prediction of the dose required to prevent restenosis requires an estimate of the proportion of surviving SMCs that are actually proliferating, after both arterial injury and

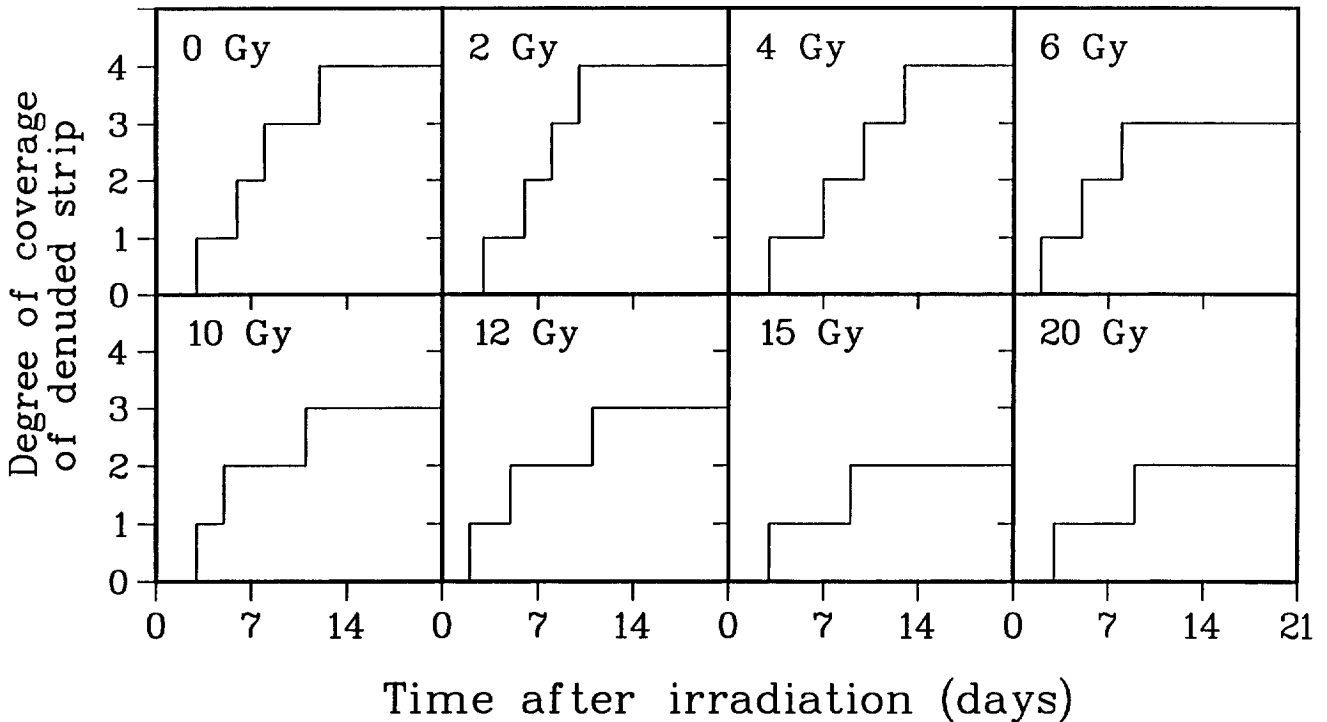


Fig. 2. The measured dose and time dependence shown by normal human aortic SMC cells *in vitro*, regarding their capacity to repopulate a 3-mm-wide denuded swathe in a 100-mm diameter petri dish which otherwise contains confluent SMCs: Degree 1: almost no cells in denuded region; Degree 2: cells visible in denuded region, but gap not bridged; Degree 3: denuded gap bridged, but cells are not confluent; Degree 4: denuded gap filled to confluence.

subsequent radiation exposure. In fact, both PTCA (2) and radiation (3, 5, 18, 19) will increase the proliferative rate of surviving cells and, based on their combined effects, we will assume that 1 in 15 (6.7%) of surviving SMC will be potentially proliferative (the arguments that follow will not be qualitatively altered if these estimates are off by as much as an order of magnitude).

Thus, we assume that there are $\sim 10^7$ target SMCs, although we stress again that the exact numbers are not necessary for the qualitative conclusions that follow.

Based on the linear-quadratic fit to the dose-survival data shown in Fig. 1, we can now estimate the number of cells that will survive different doses in a hypothetical arterial irradiation. A complication is the nonuniform dose distribution in a typical geometry, but this is relatively easy to take into account: In these calculations, based on the spatial dosimetry reported by Wiedermann *et al.* (24), we assume a linear radial dose fall-off of 10% across the arterial wall.

Based on these assumptions, the number of potentially clonogenic SMCs that would survive different radiation exposures can be simply calculated, and is shown in Fig. 4. The predicted result is that a dose of around 25 Gy would be needed to eliminate completely an assumed target population of 10^7 potentially clonogenic SMCs; this is a larger dose than can realistically be used, as it would be likely to produce an unacceptable risk of late sequelae. [Specifically, in the external-beam treatment of Hodgkin's

disease, conventionally fractionated doses above 30–40 Gy produce a significantly increased risk of late cardiac sequelae (1, 8, 17); at the dose rates under consideration here, and using an α/β ratio of 3 Gy (17), these doses correspond to a dose given continuously of 11–14 Gy, although, of course, intraluminal irradiation would result in a more advantageous dose distribution to the relevant late-responding tissues.]

It is probable, then, that the radiation doses applied in current investigations, both clinical (11, 26, 27) and experimental (12, 14, 16, 20–25), do not entirely eliminate the potentially clonogenic SMC population. Consequently, it is likely that the remaining SMCs will grow back and, unless they reach the end of their proliferative capacity, they will eventually cause restenosis, but at a later time than would be the case had the SMC not been radiation depopulated. We now estimate the time course of this regrowth.

To obtain an estimate of the rate of growth of the proliferating SMCs, we estimate (a) the increase in the number of SMCs required to produce restenosis, and (b) the average time, without irradiation, between PTCA and restenosis. First, we assume that restenosis occurs when the lumen is essentially filled with SMC; based on our assumed geometry, this would require an SMC population in the lumen of $\sim 5 \times 10^7$ cells, only a fivefold increase in the population of proliferating SMCs (relative to the population present preirradiation). Second, we observe

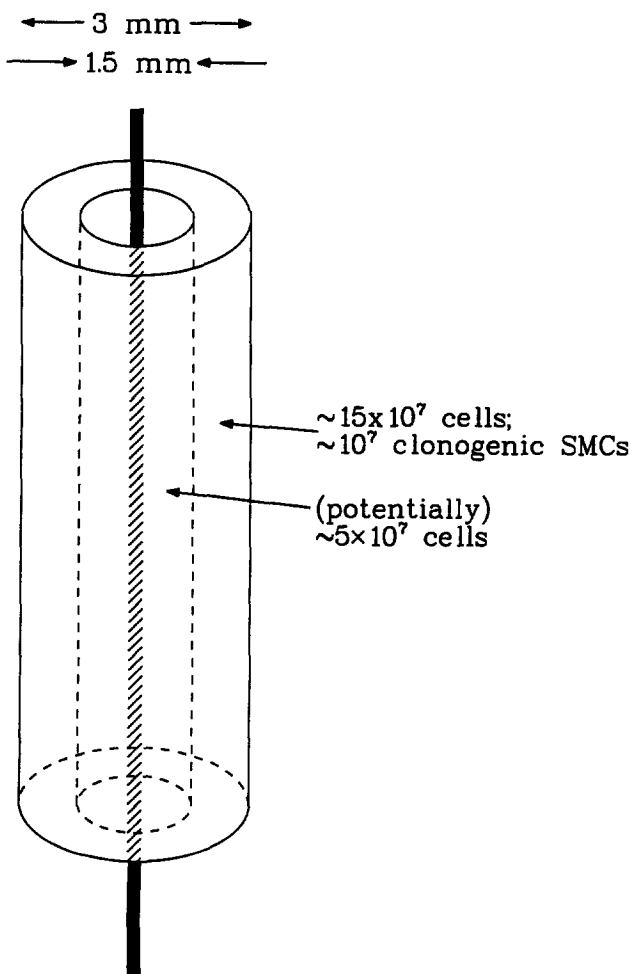


Fig. 3. Illustrating the geometry assumed here for quantitative calculations of vascular irradiation to control restenosis. The length of the irradiated artery is truncated, for clarity.

that without irradiation, restenosis is largely complete in about 4 months (13, 15). Thus, we can estimate the rate of exponential growth of SMCs during the process of restenosis to be a factor of five in 4 months, a potential doubling time of about 1.7 months. This estimate of the potential doubling time is almost certainly an upper limit, as radiation-induced accelerated repopulation is likely to shorten the effective doubling time (3, 5, 18, 19).

Having estimated the potential doubling time, we may now estimate the time for a radiation-depopulated population of SMCs to regrow to the point of causing restenosis, assuming that proliferation is not halted by senescence. This is shown in Fig. 5 for some typical maximum arterial doses. Thus, for example, this simple model suggests that a 12-Gy dose would initially cause SMC depopulation by a factor of ~1000, but that regrowth sufficient to cause restenosis would be expected in ~22 months, unless the necessary 12 population doublings are more than these cells are able to achieve before senescence. If senescence does not occur, the 12-Gy dose would postpone restenosis from about 4 to 22 months, a gain of

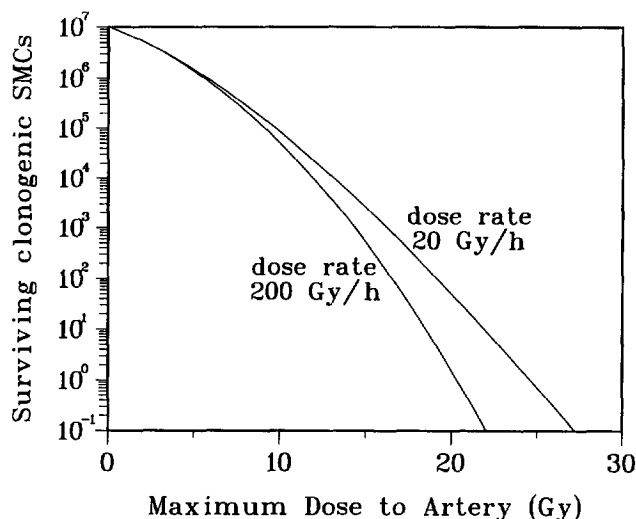


Fig. 4. Calculated number of clonogenic SMCs surviving different radiation exposures at high and intermediate dose rates. The geometry used for the calculation is that of Fig. 3, with a 10% fall-off dose across the arterial wall. The cells are assumed to follow the dose response shown by the curve in Fig. 1, with an assumed halftime for sublethal damage repair of 30 min.

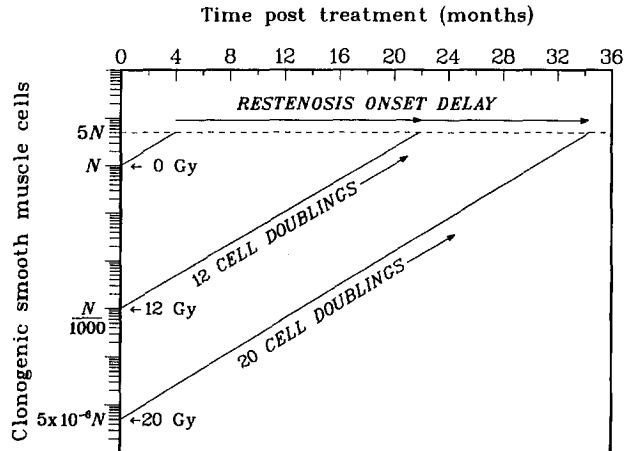


Fig. 5. Estimating the time course of restenosis onset delay. We assume that N clonogenic SMCs are present after balloon angioplasty. Their proliferation rate can be estimated by observing that without irradiation (0 Gy line), restenosis is typically complete in about 4 months, and that an increase in SMC population to about $5 \times N$ is necessary to produce restenosis. For example, the line labeled 12 Gy is drawn by estimating the initial radiation-induced SMC depopulation from the fit to the data in Fig. 1 (in this case to $N/1000$); assuming the same proliferation rate as the 0-Gy case, we draw a line parallel to the 0 Gy line until it reaches $5 \times N$, the restenosis level. Assuming that the surviving cells were capable of undergoing, in this case, 12 doublings, the time at which the line intersects this restenosis level is the time after treatment at which radiation-delayed restenosis would be predicted to occur, and this time, minus 4 months, is the delay in restenosis produced by the radiation.

18 months. Similarly, if senescence does not occur, a 20-Gy radiation dose would postpone restenosis from about 4 to 34 months, a gain of 30 months.

Recent clinical data are in general agreement with these predictions. Specifically, the Frankfurt group (11), who used an intravascular dose of 12 Gy to prevent restenosis in femoral arteries, reported no restenoses after a follow-up of 3–27 months (26), but a 16% rate after extended follow-up (27).

DISCUSSION

It is clear that the doses that would be required to eliminate completely the SMC population which has the potential to cause restenosis are too large to be practical; doses of >20 Gy delivered over a period of <1 h would be expected to result in unacceptable late complications to the artery.

However, the doses that can be practically given in vascular irradiation (≤ 20 Gy) will certainly delay restenosis by 1–3 years, with larger doses producing longer delays. Clinical results after 12 Gy do indeed show significant, but delayed, restenosis (26, 27), though whether doses below 20 Gy can avert restenosis permanently is unclear; such permanent prevention at realistic doses depends critically on the assumption that those SMCs which survive irradiation have a significantly limited capacity for proliferation.

The working rule of thumb which has emerged from our analysis is that after a maximum dose of D Gy, D population doublings are required in the surviving cells to produce restenosis. Thus, for example, after a dose of 15 Gy, if the surviving SMCs could undergo 15 doublings, they would produce restenosis.

Of course, we do not know how many doublings such SMCs can undergo before senescence, and *in vitro* studies such as those presented here are unlikely to yield quantitative answers. It has long been realized that normal tissue cells *in vivo* may have limited capabilities to divide; a figure of 50 divisions is commonly suggested (9). In an adult, many of the allowed divisions will have already been used, and so the SMCs may have a limited number of divisions available when called upon to divide after PTCA-induced damage. On the other hand, Ellis *et al.* (6) showed that restenosis rates continue to rise for up to 2 years after PTCA, suggesting that if doubling times are of the order of 2 months, SMCs in adults are still capable of significant numbers of divisions.

A further complication which we did not explicitly consider in our modeling studies is the known proliferative response of normal cells surviving high doses of radiation. It is well established that normal epithelial cells that survive radiation exposure typically undergo a proliferative response resulting in accelerated division (5, 18, 19); this is the rationale, for example, for prolonging radiotherapy to allow mucosal reactions to subside. Whether such a reaction would occur after vascular irradiation is currently unknown.

These considerations would lead to the view that it is of vital importance to perform long-term animal experiments. A dose-escalation study is needed to ascertain the relationship between dose and the length of time for which restenosis is inhibited. In addition, routine follow-up times of <1–2 years in such animal model experiments are clearly too short, and are unlikely to address some of the key mechanistic question in intravascular radiation therapy.

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