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# RADIOBIOLOGICAL PRINCIPLES IN INTRAVASCULAR IRRADIATION

Eric J. Hall, D.Sc.,* Richard C. Miller, Ph.D., and David J. Brenner, D.Sc.

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

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***Background.*** **There is wide interest in the use of intravascular irradiation to control restenosis following balloon angioplasty. Part of the mechanism of restenosis appears to be the proliferation of smooth muscle cells (SMCs), triggered to divide by the damage caused by the angioplasty, and this proliferation can be inhibited by irradiation with γ-rays or β-rays.**

***Methods. In vitro*** **data for the survival of smooth muscle cells exposed to radiation was used to model the likely control of restenosis by radiation. Physical and biological data were used to estimate differences in biological effectiveness of γ-rays and β-rays, as well as the effect on cell killing of extending the exposure time.**

***Results.*** **Based on the radiosensitivity of SMCs, measured *in vitro*, and the limited proliferative potential of these normal somatic cells, it is possible to understand and to model quantitatively how a single acute γ-ray dose in the range of 15–20 Gy can inhibit restenosis. The few successful trials carried out to date where radiation has been shown to inhibit restenosis have all involved the γ-emitter Iridium-192. The use of this radionuclide involves radiation safety problems and an inconveniently long treatment time. Consequently, there is much interest in developing a β-emitting source that would solve both problems, and a number of different possibilities are being pursued. This development introduces two new problems discussed in this paper. First, β-rays in the megavoltage range are less effective biologically than γ-rays in the kilovoltage range, but the magnitude of the difference is not well known. Second, in the case of a single large dose, such as that proposed to inhibit restenosis, the biological effect will vary substantially as the exposure time varies from 1 to 20 min. If the clinical data are to be compared between centers using a variety of β and γ-emitting radionuclides, these factors will need to be taken into account.**

***Conclusions.*** **Doses of >15 Gy are unlikely to result in elimination of the restenosis problem but should delay onset of restenosis for a significant period; the larger the dose, the longer the delay. Successful trials of endovascular radiation completed to date involve γ-rays, while many systems being developed are based on β-emitting radionuclides. Experimental data are urgently needed so that allowances can be made for the difference in dose-rate and radiation quality between γ and β-emitting radionuclides.**


***Keywords:*** **Restenosis; Intravascular; Radiation biology; Smooth muscle cells.**

## Introduction

The response of mammalian cells to ionizing radiation has been extensively studied for the past 30 years or so, since the development of techniques

*Correspondence to: Eric J. Hall, Center for Radiological Research, Columbia University, 630 West 168th Street, New York, NY 10032, USA.

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and growth media to sustain culture *in vitro*. At the doses of interest here, the dominant cellular response is cell death. The most important mechanism of radiation-induced death for the majority of cell types results from chromosomal damage, specifically the formation of exchange-type chromosomal aberrations. This mode of cell death leads to a dose–response relationship in which the fraction of cells surviving ($S$) is a linear-quadratic function of dose ($D$), *i.e.*,

$$S = e^{-\alpha D - \beta D^2}, \quad (1)$$

where $\alpha$ and $\beta$ are constants that are characteristic of a given cell type.

At low doses, the linear component dominates, but at higher doses (such as the single doses that are proposed for intravascular irradiation) the term that is quadratic in dose starts to dominate.

For some cell types, particularly those of lymphoid origin, radiation-induced apoptosis may assume considerable importance. In this mode of cell death, survival is a linear function of dose—thus, apoptotic cell death adds to the $\alpha$ component of cell lethality from the chromosome damage route. To date, there is no evidence that apoptosis is a dominant, or even an important, mechanism of radiation-induced cell death in either smooth muscle cells (SMCs) or fibroblasts.

Figure 1 shows *in vitro* cell survival data, generated in our laboratory, for SMCs of human origin for doses delivered in a single exposure. The radiosensitivity of these SMCs is unremarkable in that it falls within the range of that of most mammalian cells. For example, following a dose of 8 Gy, the fraction of cells surviving is about $10^{-2}$, *i.e.*, approximately 1 in 100 cells remain viable in the sense that they can proliferate indefinitely.

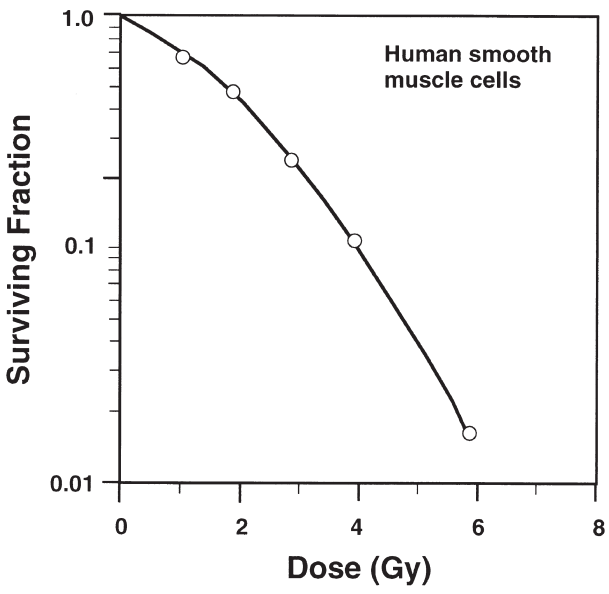


**Figure 1.** Survival data for smooth muscle cells of human origin exposed to graded doses of $\gamma$-rays. The curve represents a fit to the linear-quadratic formalism. The radiosensitivity of these cells is unremarkable and similar to many mammalian cells.

## Intravascular Irradiation to Control Restenosis

The injury caused by balloon angioplasty triggers a cascade of complex molecular events that stimulate intimal hyperplasia, as well as constrictive remodeling of the vessel wall leading eventually to recurrent narrowing of the luminal surface within the months following angioplasty. It is debatable which of these two mechanisms contributes predominantly to restenosis, but intimal hyperplasia is best understood at the molecular level, and is the mechanism that can be inhibited by radiation. The determinants of intimal hyperplasia involve the release of a host of cytokines and growth factors that in turn leads to the synthesis of gene products that stimu-


**Figure 2.** Illustration of the way in which restenosis occurs by smooth muscle cells, stimulated to divide, pouring in through a tear in the endothelial lining caused by balloon angioplasty.

late smooth muscle cell migration and proliferation. SMCs pour in through the tear in the endothelial lining that was caused by the balloon angioplasty, as illustrated in Figure 2. The use of a local agent such as radiation to inhibit cellular proliferation can therefore delay the onset of restenosis, or even prevent restenosis permanently, if a sufficiently large dose is used. Following angioplasty, there is presumably a race between the invasion of SMCs through the tear in the endothelial lining and attempts to repair that tear by the migration of endothelial cells from outside the treated area. Restenosis occurs when the SMCs win that race.

The time at which restenosis occurs in a large proportion of unirradiated individuals following balloon angioplasty (4–6 months) allows some estimate to be made of the potential doubling time of the SMCs, triggered to proliferate by damage to the endothelial lining [1, 2]. Of course, any prediction of the dose required to eliminate the clonogenic SMC population does require an estimate of the proportion of surviving SMCs that are actually proliferating, after both arterial injury and subsequent radiation exposure. In fact, both these insults will increase the proliferative rate of surviving cells [3–6] and it is based on this combined effect that we assume that 5% of surviving SMCs will be potentially proliferative. As a result of the cylindrical geometry of an artery, to a first approximation, one doubling of the smooth muscle cells would provide sufficient material to block the artery. If only 5% of the cells are clonogenic, they would be required to divide and double in number about five times to cause restenosis, *i.e.*, a five-fold increase in the population of SMCs (relative to the proliferating population present pre-irradiation) will result in restenosis. Thus, we have an estimate of the speed of exponential growth of SMCs during the process of restenosis, namely a factor of five in 4 months; a potential doubling time of a little under 2 months. This growth is shown in Figure 3 in the curve labeled "no radiation."

From our experimental data, we know the proportion of potentially clonogenic SMCs that would survive different radiation exposures. A single acute radiation dose of 12 Gy results in a depopulation of about $10^{-3}$, *i.e.*, only about 1 in 1,000 of the cells retain the ability to proliferate; these cells would need only 12 doublings to block the artery. A single acute dose of 20 Gy results in a depopulation between $10^{-5}$ and $10^{-6}$, *i.e.*, between 1 in 100,000 and 1 in 1 million cells survive. These survivors would need to double about 20 times to produce sufficient progeny to block the artery. The bigger the dose, the longer the delay in restenosis. If it takes 4–6

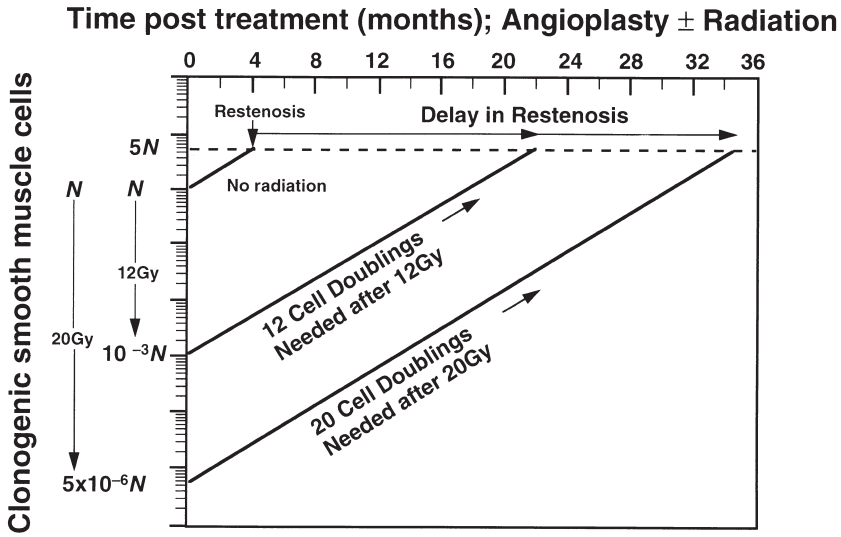


**Figure 3.** Estimating the time course of the delay of restenosis onset. We assume that $N$ clonogenic smooth muscle cells (SMCs) are present after balloon angioplasty. Their proliferation rate can be estimated by observing that, without irradiation, restenosis is typically complete in about 4 months, and that an increase in SMC population to about $5 \times N$ is necessary to produce restenosis. Thus the slope of the line labeled "no radiation" gives the appropriate cellular proliferation rate. The line labeled, for example, "12 Gy" is then drawn by estimating the initial radiation-induced SMC depopulation ($10^{-3}$); assuming the same proliferation rate as the "no radiation" case, we draw a line parallel to the no radiation line, until it reaches $5 \times N$, the restenosis level. The time at which the line intersects the restenosis level ($5 \times N$) is the time, posttreatment, 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. 20 Gy causes a greater depopulation (to $5 \times 10^{-6}$) and consequently a longer delay of restenosis.

months for restenosis to occur without radiation, it may take 2 years or more for restenosis after a sizeable dose of radiation. However, these SMCs are normal somatic cells, they are *not* malignant and therefore do not have the capacity for indefinite proliferation. Normal somatic cells senesce after 40–60 cell divisions in a young person, and after far fewer divisions (perhaps 15–20 divisions) in an elderly person [7]. With each division the telomeres on the chromosome ends shorten. Twenty divisions are required to make good the depopulation resulting from a dose of 20 Gy. A dose of this magnitude, therefore, may result in a permanent inhibition of restenosis, because cells would not be capable of this many divisions.

Overall, we can conclude that (a) the beneficial effect of endovascular irradiation is primarily related to the cell killing (and consequent population reduction) of SMCs, which would otherwise initiate neointimal hyperplasia, and (b) there is not a large safety margin between the radiation dose required for the long-term control of restenosis (~20 Gy) and the radiation dose that might result in undesirable late sequelae (~30 Gy).

## Choice of Radioisotopes for Endovascular Brachytherapy

The few successful trials carried out to date in which radiation has been shown to inhibit restenosis in humans or pigs have all involved the γ-emitter Iridium-192. The use of this radionuclide introduces two major problems:

1. Radiation safety: Because of the penetration of the γ-rays involved, the patient must be in some sort of shielded room during treatment in order to protect staff.
2. Treatment time: Limitations of specific activity dictate that treatment times to deliver a dose of 15–20 Gy at about 3 mm from the source are about 20 min.

Both of these problems would be solved if a β-emitting radionuclide were used. Because of the limited range of β-rays, treatments could be carried out in the cardiology laboratory with minimal radiation protection, and treatment times could be on the order of a few minutes. For this reason, much commercial effort is focused on developing a practical system based on a β-emitting radionuclide. On the other hand, the short range of the β-rays makes them extremely sensitive to problems of source centering, which may well limit their eventual usefulness. Table 1 shows the sources that are under active consideration.

## Dose–Rate Effect in Radiation Biology

The biological effect of a given dose of low linear energy transfer (LET, sparsely ionizing) radiation depends critically on the dose–rate at which it is delivered or, put another way, on the total exposure time [8]. This has been known from clinical observations in brachytherapy from the 1940s, and was demonstrated in detail with cells in culture by Hall and Bedford in the 1960s [9]. This phenomenon has come to be known as the dose–rate effect and is one of the most important factors that determine the fraction of cells killed by a given dose of x-rays, γ-rays, or electrons.

The magnitude of the dose–rate effect depends on the cell type, being largest for cells that characteristically die a mitotic death and smallest for cells that die principally an apoptotic death [10]. The dose–rate range, or range of exposure times, over which the dose–rate effect is important, is determined by the rate of repair of sublethal damage, which is quantified by the half-time for sublethal damage repair ($T_{1/2}$). Brenner and Hall [11] reviewed the literature for around 40 cell lines of human origin, cultured *in vitro*, and found a wide range of $T_{1/2}$ values, approximately log-normally distributed, with a geometric mean value of about 16 min.

## Dose–Rate Effect and Its Influence on Vascular Brachytherapy to Prevent Restenosis

The beneficial effect of endovascular irradiation is related to the cell killing (and consequent population reduction) of SMCs, which would otherwise

**Table 1.** Possible isotopes for intraluminal brachytherapy

| Isotope | Emission | Maximum energy (keV) | Average energy (keV) | Half-life | Activity |
|---|---|---|---|---|---|
| Ir-192 | Gamma | 612 | 375 | 74 days | 1.0 Ci |
| I-125 | X-ray | 35 | 28 | 60 days | 3.8 Ci |
| Pd-103 | X-ray | 21 | 21 | 19 days | 3.9 Ci |
| P-32 | Beta | 1710 | 690 | 14 days | 40 mCi |
| Sr/Y-90 | Beta | 2270 | 970 | 28 years | 30 mCi |
| W/Re-188 | Beta | 2130 | 780 | 69 days | 35 mCi |

initiate neointimal hyperplasia. There is not a large safety margin between the radiation dose required for the long-term control of restenosis (~20 Gy) and the radiation dose that might result in undesirable late sequelae (~30 Gy), so that it is crucial to optimize the prescribed dose for restenosis prevention, and then to be able to deliver the radiation dose that is isoeffective to this optimized dose, even when the dose–rate changes significantly. The various techniques used or proposed for the delivery of the radiation dose include γ- and β-emitters, most of which have relatively short physical half lives. The variation in exposure times over the practical lifetime of a given radioactive source are on the order of the half-time of repair of sublethal damage characteristic of human cells, so that the resultant biological effect will be critically dependent on the dose–rate/exposure time.

Thus, the dose–rate effect is a biological factor that will need to be taken into account on a daily basis in clinical practice, because almost all of the radionuclides proposed for use have relatively short half-lives. For example, iridium-192 has a half-life of 60 days and sources are usually replaced after about two half-lives; thus, the exposure times will vary by a factor of four during the practical lifetime of the sources, which will have a profound effect on the biological effectiveness of a given prescribed dose of about 20 Gy. As another example, a β-emitting source of $^{90}$Y (radioactive half life 64 h) has a useful lifetime of about 1 week, during which the dose–rate will vary by a factor of six, with consequent significant impact on the biological effectiveness of a given prescribed dose. Figure 4 shows the results of calculations of the doses equivalent to 20 Gy delivered as an acute exposure lasting 2 min, for exposure times up to 25 min and for $T_{1/2}$ values of 15–45 min. A short exposure time of 1 or 2 min is possible with a β-emitting radionuclide. The longer and more variable exposure times up to 25 min are characteristic of γ-emitting radionuclides, such as iridium-192. It is evident that extending the exposure time has a substantial effect; 20 Gy delivered in 25 min is equivalent to only 17–19 Gy delivered in 1 min. If clinical results between patients are to be compared, particularly from institutions that use different radionuclides, this dose–rate effect must be taken into account.

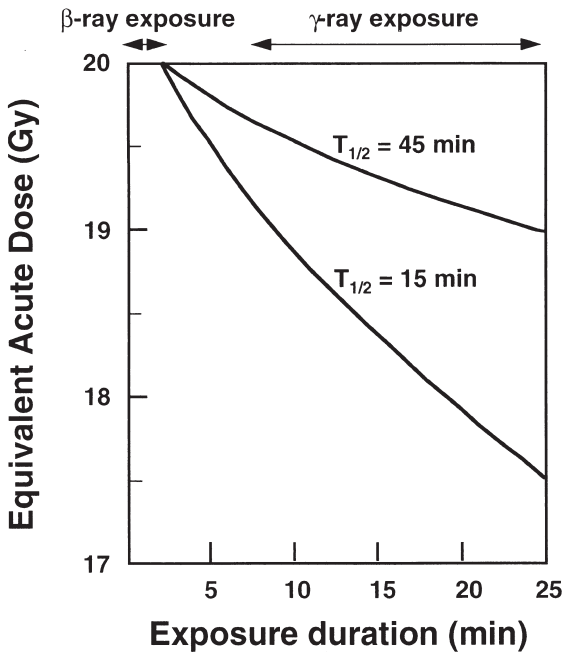


**Figure 4.** Equivalent acute doses to actual doses, delivered in exposure times up to 25 min. Two possible values are assumed for the half-time of repair of sub-lethal damage, namely 15 and 45 min. No experimental data are available for repair times in smooth muscle cells of human origin.

## Radiation Quality and Its Influence on Vascular Brachytherapy to Prevent Restenosis

There is good radiobiological evidence that electrons in the megavoltage range are less effective biologically than kilovoltage x- or γ-rays. The microdosimetric analysis also suggests a relative biologic effectiveness of less than unity, based on the different physical energy deposition properties associated with the two types of radiation [12]. Specifically, the average photon energy from a highly filtered 250 kVp x-ray beam is ~85 keV [13] and the average electron energy set in motion by the interaction of these photons with tissue is ~20 keV [14]. These energies are much lower than those corresponding to either megavoltage photons or to β-rays emitted by radionuclides proposed for intravascular brachytherapy; those have an average electron energy of several hundred kilovolts and a maximum electron energy close to 1 MeV. This would imply a significant relative biologic effectiveness difference, which could be 0.6 at very low doses and perhaps 0.85 at high therapeutic doses. This subject has been discussed at length in a recent paper by Brenner [15].

In summary, the early trials that have demonstrated the effectiveness of endovascular brachytherapy to inhibit restenosis have all involved the γ-emitter iridium-192. A great variety of systems are under development, driven largely by the technology. There is a lack of knowledge of the relative ef-

fectiveness of β-rays and γ-rays, and little knowledge of the influence of a time factor for overall treatment time between 60 s and 20 min, which is likely to be substantial when a single acute dose of 20 Gy is involved. Experimental data are urgently required to answer these questions and provide guidance for future clinical trials.

## Conclusions

The actual numbers emerging from this simple model of radiation-induced restenosis delay are only approximate. However, the results to date indicate that:

1. Routine follow-up of less than 1 year in animal experiments investigating the effect of radiation on restenosis results in an experimental design that is unlikely to detect radiation-delayed restenosis.
2. Doses of <15 Gy are unlikely to result in elimination of the restenosis problem but should delay onset of restenosis for a significant period; the larger the dose, the longer the delay.
3. There appears to be a relatively narrow window between the minimum dose needed to slow down the proliferation of SMCs and the maximum dose that can be tolerated before late sequelae occur in the vessel wall.
4. Successful trials of endovascular radiation completed to date involve γ-rays, whereas many systems being developed are based on β-emitting radionuclides. Experimental data are urgently needed so that allowances can be made for the difference in dose–rate and radiation quality between γ- and β-emitting radionuclides.

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