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Biological Response to Nonuniform Distributions of ^{210}Po in Multicellular Clusters

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Abstract

Radionuclides are distributed nonuniformly in tissue. The present work examined the impact of nonuniformities at the multicellular level on the lethal effects of ^{210}Po . A three-dimensional (3D) tissue culture model was used wherein V79 cells were labeled with ^{210}Po -citrate and mixed with unlabeled cells, and multicellular clusters were formed by centrifugation. The labeled cells were located randomly in the cluster to achieve a uniform distribution of radioactivity at the macroscopic level that was nonuniform at the multicellular level. The clusters were maintained at 10.5°C for 72 h to allow α -particle decays to accumulate and then dismantled, and the cells were seeded for colony formation. Unlike typical survival curves for α particles, two-component exponential dose-response curves were observed for all three labeling conditions. Furthermore, the slopes of the survival curves for 100, 10 and 1% labeling were different. Neither the mean cluster absorbed dose nor a semi-empirical multicellular dosimetry approach could accurately predict the lethal effects of ^{210}Po -citrate.

INTRODUCTION

Radionuclides that emit α particles are ubiquitous in nature; they are also produced for use in a variety of applications. The short range (20–90 μm in soft tissue), high LET, and dose-rate independence of their biological effects make α particles a desirable radiation for targeted medical therapies. Understanding and predicting biological responses to α -particle emitters is essential for both risk assessment and therapeutic application of radionuclides. However, predicting biological responses to radionuclides is complicated. When radionuclides are ingested or administered, they are generally distributed nonuniformly at the organ, suborgan, multicellular, cellular and subcellular levels (1). The resulting nonuniform dose distributions can have a profound impact on the biological response. Hence dosimetry at both the macroscopic and microscopic levels is important.

Extensive theoretical and experimental investigations have been carried out to examine the impact of nonuniform distributions of α -particle emitters on cell survival curves (2-6). While theoretical models can exert a high degree of control over the variables to be investigated, such control is difficult to achieve in laboratory studies of the biological effects of radioactive materials, particularly in 3D tissue models. Some studies have assessed the radiotoxicity of α -particle emitters in multicellular spheroids (7-9). These studies provide much-needed insight into the capacity of α -particle-emitting radiopharmaceuticals to sterilize micrometastatic lesions. However, the use of spheroids in the development and refinement of multicellular dosimetry model is limited, because one has very little control over the distribution of

radioactivity. Penetration of the radiopharmaceutical is often limited because of diffusion, surface binding, etc. (10). This can be circumvented by assembling multicellular clusters of radiolabeled and unlabeled cells (11). The purpose of the present work was to explore the lethality of an α -particle emitter under conditions where the distribution of radioactivity is well controlled. The resulting data can be used in the further development of theoretical models that predict the biological effects of nonuniform distributions of radionuclides that emit α particles.

The α -particle emitter ^{210}Po is found in nature and, when ingested by animals, is readily detectable in a variety of organs (12). It has a physical half-life of 138.4 day and emits a single 5.3 MeV α particle, making it an excellent candidate for laboratory investigations. In the present work, the effects of nonuniform distributions of ^{210}Po were studied in an *in vitro* multicellular cluster model that enabled tight control of the variables affecting distribution of radioactivity at the multicellular level. This was accomplished by assembling multicellular clusters containing 4×10^6 cells wherein 100, 10 or 1% of the cells contained ^{210}Po -citrate. This allowed us to maintain a uniform distribution at the macroscopic level while altering the nonuniform distribution at the multicellular level. The 5.3 MeV monoenergetic α particles cross-irradiate about four cell diameters. Accordingly, the labeled cells receive both self- and cross-dose, whereas the unlabeled cells receive only cross-dose. The cells irradiated in the cluster are then disassembled, and the clonogenic surviving fraction (SF) is determined. The SF is correlated with the mean absorbed dose to the cluster at the macroscopic level and with the mean self- and cross-doses to the cells at the microscopic level.

MATERIALS AND METHODS

Cells

Chinese hamster V79 lung fibroblasts were used in the present study, with clonogenic survival as the biological end point. The different minimum essential media (MEMA, MEMB and wash MEMA) and culturing conditions have been described elsewhere (11,13).

Radiochemical and Quantification of Radioactivity

^{210}Po (74 MBq/ml) was obtained as PoCl_4 in 2 M HCl from Isotope Products (Valencia, CA). ^{210}Po -citrate was prepared by mixing the stock ^{210}Po solution with 1 M sodium citrate at a ratio of 1:7, resulting in a pH of 5.8. The ^{210}Po -citrate was diluted with MEMB in different ratios (referred to as recipes). In the case of 100% and 10% labeling conditions, ^{210}Po -citrate was mixed with MEMB at a ratio of 1:19 to obtain ~36 kBq/ml. The 1% labeling condition was mixed at a ratio of 1:1 with MEMB to achieve ~160 kBq/ml. The final pH of the solution in both recipes was 6.9. These recipes were similar to those in ref. (14). The ^{210}Po activities were determined with a Beckman LS5000 automatic liquid scintillation counter by transferring aliquots of radioactive culture medium into 5 ml of Ecolume liquid scintillation cocktail (LSC) from MP Biomedical (Aurora, OH) in triplicate. The detection efficiencies for the ^{210}Po 5.3 MeV α particles in 30 μl of MEMB and 500 μl of cell suspensions in MEMB were 0.7 and 0.5, respectively.

Assembly of Multicellular Clusters

Multicellular clusters containing nonuniform distributions of ^{210}Po were assembled according to protocols described in detail elsewhere (11,15). Briefly, V79 cells were washed with 20 ml PBS, trypsinized with 0.05% trypsin-0.53 mM EDTA, and suspended at 4×10^5 or 4×10^6 cells/ml in MEMB. Aliquots of 1 ml were placed in round-bottom culture tubes and placed on a rocker-roller (Fisher Scientific, Springfield, NJ) for 12–14 h at 37°C in an atmosphere of 95% air and 5% CO_2 . One milliliter of MEMB containing various activities of ^{210}Po -citrate was then added to one set of tubes (denoted “labeled”). One milliliter of MEMB was added to the second set of tubes (denoted “unlabeled”), and the tubes were returned to the rocker-roller.

After 0.5 h the cells were washed, resuspended and passed through a 21-G needle. The labeled cells were then mixed with unlabeled cells to get 100, 10 or 1% radiolabeled cells in a total population of 4×10^6 cells (see Table 1) and centrifuged. The pellets were transferred to a sterile 400- μ l polypropylene microcentrifuge tube, centrifuged to form clusters, and maintained at 10.5°C. The cells accumulated the preponderance of their radioactive decays while in clusters as opposed to the radiolabeling and colony-forming periods.

Determination of Surviving Fraction (SF) of Cells in the Multicellular Clusters

After 72 h at 10.5°C, the cells were transferred to 17 $\times$ 100-mm Falcon polypropylene tubes, washed, resuspended in MEMA, passed through a 21-G needle, and serially diluted, and 1 ml of the appropriate dilutions was seeded for colony formation (11). Cell counts before and after assembly of the clusters showed that essentially all 4×10^6 cells in the cluster were recovered. Aliquots were taken from each tube before dilution and the mean radioactivity per labeled cell was determined (Table 1). After 1 week, the colonies were stained and scored (11). The SF compared to parallel control was determined for each radioactivity concentration.

Toxicity of Citrate

Studies were carried out to ensure that the citrate concentrations used in the labeling procedures did not result in chemotoxicity by preparing control solutions with non-radioactive 2 M HCl. Cells were manipulated identically to those in the ^{210}Po -citrate studies. No citrate toxicity was observed over the range of concentrations used.

Cell Viability

Aliquots of labeled cell suspensions that were exposed to different activities were mixed in a ratio of 1:1 with 4% trypan blue solution (Fluka Chemie, Buchs). The selected concentrations of 0, 0.51, 3.8 and 67 kBq/ml correspond to the maximum concentrations used in the present study (Table 1) in each recipe. Aliquots of cells were immediately loaded into a hemocytometer and viewed at 100 $\times$ magnification with an upright Nikon Labophot II microscope. The number of viable cells (trypan blue negative) and dead cells (trypan blue positive) were scored in about 3000 cells. The percentages of dead cells in the populations labeled at 0–67 kBq/ml ranged from 3.0–3.1% (16). The remaining cell suspensions at all the concentrations were kept rolling at 10.5°C. After 72 h rolling, cell viability was determined as described above; the percentage of nonviable cells remained the same.

Assessment of Feeder Effect

Further studies were carried out to determine whether the seeding density influenced the plating efficiency (17). About 3×10^6 cells were suspended in MEMA in a 17 $\times$ 100-mm tube and subjected to 60 Gy of ^{137}Cs γ rays (26 Gy/min). Aliquots of 150,000 lethally irradiated feeder cells (100 μ l) were plated in triplicate into culture dishes containing 4 ml MEMA along with 200 unlabeled cells, 200 labeled cells, or no additional cells. The presence of the feeder cells had no impact on the SF.

Mean Cellular Absorbed Doses

The biological response of the cluster is dictated by contributions to the absorbed dose from radiations emitted from both labeled and unlabeled cells. It is important to account for all absorbed dose contributions that occur during the 0.5-h incubation (I) on the rocker-roller where the radioactivity is taken up by the cells, the 72-h maintenance (M) at 10.5°C, and the 1-week colony-forming period (CF). The mean absorbed dose to the nucleus of any given labeled cell in the cluster arises from: (a) self-dose from decays within the labeled cell that occur during I , $D_{\text{self},I}^{\text{labeled}}$; (b) self-dose from decays within the labeled cell during M , $D_{\text{self},M}^{\text{labeled}}$; (c)

self-dose from decays within the labeled cell during CF , $D_{\text{self},CF}^{\text{labeled}}$; (d) cross-dose from decays occurring in the extracellular medium during I , $D_{\text{cross},I}^{\text{labeled}}$; (e) cross-dose from decays in neighboring labeled cells in the cluster, $D_{\text{cross},M}^{\text{labeled}}$; (f) cross-dose from decays in neighboring progeny in the colony, $D_{\text{cross},CF}^{\text{labeled}}$. In contrast, the only absorbed dose received by unlabeled cells is the cross-dose from decays in neighboring labeled cells in the cluster, $D_{\text{cross},M}^{\text{unlabeled}}$. Calculation of the absorbed dose from each of these contributions is addressed below.

Cellular Self-Absorbed Dose to Labeled Cells (self-dose)

The cumulated activities during the three periods were determined as described earlier (11, 13), with the exception of the incubation time $t_I = 0.5$ h during which the radioactivity was taken up by the cells (18), the physical half-life $T_p = 138.4$ days for ^{210}Po , and the biological clearance half-time $T_b = 13.8$ h for ^{210}Po -citrate (14). If A_0^{labeled} is the cellular uptake at the end of the uptake period, then, using the prescription and notation in the Appendix of ref. (13), the cellular activities are given by

$$A_I^{\text{labeled}}(t) = \kappa t \quad (1)$$

$$A_M^{\text{labeled}}(t) = A_0^{\text{labeled}} e^{-0.693t/T_p} \quad (2)$$

$$A_{CF}^{\text{labeled}}(t) = A_0^{\text{labeled}} e^{-0.693t_M/T_p} e^{-0.693t/T_p} \quad (3)$$

The cumulated activities during these periods are given by

$$\tilde{A}_I^{\text{labeled}} = \int_0^{t_I} A_I^{\text{labeled}}(t) dt = 0.5 t_I A_0^{\text{labeled}} \quad (4)$$

$$\tilde{A}_M^{\text{labeled}} = 1.44 T_p A_0^{\text{labeled}} \left(1 - e^{-0.693t_M/T_p}\right) \quad (5)$$

$$\tilde{A}_{CF}^{\text{labeled}} = 1.44 A_0^{\text{labeled}} T_e e^{-0.693t_M/T_p} \left(1 - e^{-0.693t_{CF}/T_e}\right) \quad (6)$$

The temporal dependence of cellular activity and cumulated activity during the three distinct periods are represented graphically in Fig. 1.

Following the general formalism [Eq. 7 of ref. (19)], the mean self-absorbed dose to the nuclei of the labeled cells, $D_{\text{self}}^{\text{labeled}}$, is given by

$$D_{\text{self}}^{\text{labeled}} = \tilde{A} \left[f_N S(N \leftarrow N) + f_{Cy} S(N \leftarrow Cy) \right], \quad (7)$$

where $\tilde{A} = \tilde{A}_I + \tilde{A}_M + \tilde{A}_{CF}$. $S(N \leftarrow N)$ and $S(N \leftarrow Cy)$ are the absorbed dose to the nucleus per unit cumulated activity in the nucleus and cytoplasm, respectively. f_N and f_{Cy} fraction of cellular radioactivity in the nucleus and cytoplasm, respectively. The radii of the cell and nucleus of V79 cells are 5 μm and 4 μm (14), respectively. The subcellular distribution of ^{210}Po -citrate is $f_{Cy} = 0.72$ and $f_N = 0.28$ (16,18). The values for $S(N \leftarrow N)$ and $S(N \leftarrow Cy)$ for V79 cells with the above-mentioned dimensions are 1.55×10^{-1} and 7.12×10^{-2} Gy Bq $^{-1}$ s $^{-1}$, respectively (19). Substitution of relevant parameters into Eq. (7) yields a mean self-absorbed dose to the nucleus of the labeled cells of 31 Gy per mBq per labeled cell.

Cross-Absorbed Dose to the Labeled and Unlabeled Cells (cross-dose)

For ^{210}Po , the mean cross-dose constitutes the majority of the mean dose delivered to the labeled and unlabeled cells. For the I period, the cross-dose to the labeled cells, $D_{\text{cross},I}^{\text{labeled}}$, is approximately the mean absorbed dose to the culture medium, D_I^{medium} , whereas for M , $D_{\text{cross},M}^{\text{labeled}}$ is approximately the mean absorbed dose to the cluster, D_M^{cluster} . In general (20), these are given by

$$D_{\text{period}}^{\text{target}} = \tilde{A}_{\text{period}}^{\text{source}} \Delta \phi (\text{target} \leftarrow \text{source}) / m^{\text{target}}, \quad (8)$$

where $\tilde{A}_{\text{period}}^{\text{source}}$ is the cumulated activity in the source region during the source period, m^{target} is the mass of the target region, Δ is the mean energy emitted per nuclear transition, and ϕ is the fraction of the energy emitted from the source region that is absorbed in the target region (20). When recoil energy is neglected for ^{210}Po , $\Delta = 8.5 \times 10^{-13}$ Gy kg Bq $^{-1}$ s $^{-1}$ (21).

To calculate D_I^{medium} from decays in the medium (*e.g.* medium $\leftarrow$ medium), the mass is taken as 2 g and $\phi \sim 1$ for α -particle radiations since they are considered to be fully absorbed by the medium due to their short range. Assuming no physical decay, $\tilde{A}_I^{\text{medium}} \sim A_I^{\text{medium}} t_I$. Thus substitution into Eq. (8) gives $D_{\text{cross},I}^{\text{labeled}} \sim D_I^{\text{medium}} = 0.00153$ Gy per kBq/ml. Using the slope of the cellular uptake period of 0.028 (100% or 10%) and 0.116 (1%) mBq/cell per kBq/ml (see the Results), this can be expressed as $D_I^{\text{medium}} = 0.055$ and 0.013 Gy per mBq/cell, respectively. Unlabeled cells are not irradiated during the uptake period.

The mean cross-dose received by the labeled, $D_{\text{cross},M}^{\text{labeled}}$, and unlabeled, $D_{\text{cross},M}^{\text{unlabeled}}$, cells during period M can be approximated by the mean cluster dose, D_M^{cluster} . The cumulated activity can be obtained from Eq. (5) simply by replacing “labeled” with “cluster”. The mass of a cluster of V79 cells containing 4×10^6 cells is 7.9 ± 0.1 mg (11). With $\phi \sim 1$, substitution of required parameters into Eq. (8) gives $D_M^{\text{cluster}} = 28.2$ Gy/kBq. This translates to 112.8 Gy per mBq per labeled cell for 100% labeling. The mean cross-doses scale to 11.3 and 1.1 Gy per mBq per labeled cell for 10 and 1% labeling, respectively.

Finally, the cross-dose received by the labeled cells during CF arises from neighboring cells in the colony. The cross-dose cellular S values, $S_{\text{cross}}(N \leftarrow N)$ and $S_{\text{cross}}(N \leftarrow Cy)$, for V79 cells are 1.03×10^{-2} and 1.09×10^{-2} Gy Bq $^{-1}$ s $^{-1}$, respectively (22). As an upper limit, when one considers the increase in surrounding neighbors and decrease in activity per cell due to proliferation, the cross-dose to the nucleus during CF period $D_{\text{cross},CF}^{\text{labeled}}$, can be approximated as

$$D_{\text{cross},CDF}^{\text{labeled}} \approx 1.44T_p \left(1 - e^{-0.693t_{CF}/T_F}\right) \frac{A_0^{\text{labeled}}}{2} \times \left[f_N S_{\text{cross}}(N \leftarrow N) + f_{Cy} S_{\text{cross}}(N \leftarrow Cy) \right]. \quad (9)$$

Substitution of the required parameters into Eq. (9) yielded a mean cross-dose to the nucleus during *CF* of 2.9 Gy/mBq per labeled cell. This value is valid irrespective of the percentage of labeled cells because it is expressed as the cross-dose per unit activity in the labeled cells. Unlabeled cells receive no significant cross-dose due to the substantial distances between cells seeded for *CF*.

Semi-empirical Model

Recently, a semi-empirical multicellular dose–response model for a mixed population of labeled and unlabeled cells was validated for the case of ^{131}I after isolating the effects of self-dose (13). Briefly, if f represents the fraction of cells in the tissue that are labeled and $(1 - f)$ is the fraction of cells that are unlabeled, then the SF of the mixed population of cells, SF^{mixed} , is (13)

$$\text{SF}^{\text{mixed}} = f \text{SF}^{\text{labeled}} + (1 - f) \text{SF}^{\text{unlabeled}}, \quad (10)$$

where $\text{SF}^{\text{labeled}}$ and $\text{SF}^{\text{unlabeled}}$ are the fractions of labeled and unlabeled cells that survive, respectively. In the present work, we expand on our previous relationship by specifically considering the self- and cross-doses to both labeled and unlabeled cells during all periods of the experiment. Assuming exponential dose responses to self- and cross-doses, it can be shown that

$$\text{SF}^{\text{unlabeled}} = \exp\left(-D_{\text{cross}}^{\text{unlabeled}}/D_{37,\text{cross}}\right), \text{SF}^{\text{labeled}} = \exp\left(-D_{\text{self}}^{\text{labeled}}/D_{37,\text{self}}\right) \exp\left(-D_{\text{cross}}^{\text{labeled}}/D_{37,\text{cross}}\right), \quad (11)$$

where $D_{\text{self}}^{\text{labeled}} = D_{\text{self},I}^{\text{labeled}} + D_{\text{self},M}^{\text{labeled}} + D_{\text{self},CF}^{\text{labeled}}$, $D_{\text{cross}}^{\text{labeled}} = D_{\text{cross},I}^{\text{labeled}} + D_{\text{cross},M}^{\text{labeled}} + D_{\text{cross},CF}^{\text{labeled}}$ and $D_{\text{cross}}^{\text{unlabeled}} = D_{\text{cross},M}^{\text{unlabeled}}$. The quantities $D_{37,\text{self}}$ and $D_{37,\text{cross}}$ are the mean self- and cross-doses required to produce 37% survival.

RESULTS

Uptake of ^{210}Po -citrate in V79 cells

As in previous studies (14,23), the cellular uptake of ^{210}Po -citrate was linear with the concentration of ^{210}Po -citrate in culture medium over the period *I* (Fig. 2). A linear least-squares fit to the data for each experiment (Fig. 2) produced the slopes given in the second column of Table 2. The slopes for 100% labeling are lower than for 10% labeling (Table 2). Furthermore, the slopes for 1% are quite high compared to those for 10%, although 4×10^5 cells were labeled in both cases. This may be due to the different recipe used for preparation of the higher activity concentrations needed for the 1% cluster. The data in Table 2 also suggest that the slope may be related to the age of the ^{210}Po -citrate stock solution and the number of times the cells have been subcultured (flask passage number). The variations in slope are not an impediment for the present study, because the SF is correlated with the measured cellular activity (mBq/cell) and not the extracellular concentration (kBq/ml).

Response of Multicellular Clusters to ^{210}Po -citrate

Figure 3 shows the SF of cells in the multicellular cluster as a function of the ^{210}Po activity per labeled cell. The composite data for each labeling condition were least-squares fitted by a two-component exponential function,

$$\text{SF} = (1 - b) e^{-A/A_1} + b e^{-A/A_2}, \quad (12)$$

where A is the activity per labeled cell and b , A_1 and A_2 are the fitted parameters. The values of the fitted parameters are summarized in Table 3. In general, fitting all three parameters at the same time did not lead to fits that represented the data adequately. Accordingly, curve fits were carried out with a two-step approach. First, the data in the second component of the survival curve were selected and a linear least-squares fit of $\log \text{SF}$ as a function of A was performed to obtain A_2 and b . These parameters were then fixed and the entire data set was fitted to Eq. (12) to arrive at A_1 . In one instance, A_1 was obtained in the same manner as A_2 .

It is also instructive to plot the SF as a function of cluster activity (kBq) (Fig. 4). These data were least-squares fitted with Eq. (12); the resulting fitted parameters are summarized in Table 3. Using Eq. (8), the mean absorbed dose to the cluster during M was 28.2 Gy/kBq of cluster activity. An additional 2.4 Gy/kBq is delivered to the cells during CF in the case of 100% labeling. This contribution was not considered for 10% and 1% labeling because the survival curves primarily represent the response of unlabeled cells that receive no dose during CF . Figure 5 shows the SF as a function of mean absorbed dose to the cluster for ^{210}Po -citrate. For comparison, historical survival curves are also shown for ^{131}I iododeoxyuridine (^{131}IdU , β particles) and chronic ^{137}Cs γ rays from experiments in which the clusters were exposed to the respective radiations for 72 h under similar conditions. This figure illustrates the RBE of the different radiations for cell killing in V79 multicellular clusters. The mean lethal cluster doses at 37% survival (D_{37}) are given in Table 4 for each labeling condition along with comparative historical data for chronic ^{137}Cs γ rays and ^{131}IdU .

Semi-empirical Model

Multicellular dosimetry provides the mean cellular self- and cross-doses for labeled and unlabeled cells during each period of the experiments (Table 5). For 100% labeling, the total cross-dose to the labeled cell is 115.8 Gy per mBq per labeled cell, the majority coming from M . I and CF together constitute <3% of the total mean cross-dose for 100% labeling. Labeled cells also receive a self-dose of 31 Gy per mBq per labeled cell. As expected, the mean self-dose per mBq in the labeled cell is independent of the percentage of cells labeled, whereas the cross-dose decreases (i.e., the same mBq per cell in fewer cells leads to lower cluster activity and therefore a lower cross-dose). Using these data, the survival data in Fig. 3 are replotted in Fig. 6 as a function of both mean self-dose to labeled cells (bottom abscissa) and mean cross-dose to unlabeled cells (top abscissa). Note that the magnitude of the self-dose axes vary ten-fold between each successive labeling condition (100%, 10%, 1%) though the scale of the cross-dose axes remains the same. Figure 6 illustrates the response as modeled with the semi-empirical function (Eq. 10). The $D_{37,\text{self}}$ of 0.68 Gy was adopted from our earlier studies with ^{210}Po -citrate in V79 cell suspensions (maintained at 10.5°C) where only self-dose was delivered (23). Ideally, one should obtain $D_{37,\text{self}}$ directly from the present experimental data with cell sorting (13). However, this was not possible because regulatory limitations precluded sorting cells containing ^{210}Po . $D_{37,\text{cross}}$ was assumed to be equal to the mean lethal cluster dose (D_{37}) of 0.64 Gy obtained for 100% labeling. This mean lethal dose was used because, for 100% labeling, theoretical multicellular dosimetry for ^{210}Po indicated that about 90% of the total dose received by any given cell is cross-dose (22). The self- and cross-doses used in the semi-empirical model were taken from Table 5. The Fit Comparison tool (Originlab Corp.,

Northampton, MA) was used to determine how well the semi-empirical model matched the best least-squares fit to the experimental data (24). The resulting P values obtained from an F test are 0.93, 0.98 and 0.79 for 100, 10 and 1% labeling, respectively.

DISCUSSION

The lethal effects of α particles have been studied extensively. In general, uniform irradiation of mammalian cells with α particles results in clonogenic cell survival curves that fall exponentially with the absorbed dose and have no shoulder. Such survival curves are similar to those induced by high-LET radiation (25). In keeping with this, the first two logs of the survival curves for ^{210}Po -citrate-labeled cells in 3D multicellular clusters are also exponential (Fig. 5). In the case where 100% of the cells were labeled with ^{210}Po -citrate, a mean D_{37} of 0.64 Gy was obtained (Table 4). This value is within experimental uncertainties of the value found for monolayers of the same cell line (14,18,23). When compared to the D_{37} for chronic ^{137}Cs γ rays (11), the RBE for the ^{210}Po α particles is 19.1. A comparison with the response to acutely delivered ^{137}Cs γ rays that have a D_{37} of 6.4 Gy (15) yields an RBE of 10. These RBEs are within the standard errors of the reported values of 16 (^{210}Po compared to chronic $^{99\text{m}}\text{Tc}$ γ rays) and 6 (^{210}Po compared to acute ^{137}Cs γ rays) that were obtained when suspensions of V79 cells were similarly labeled with ^{210}Po -citrate and immediately plated for colony formation (14). Various groups have reported RBE values for α particles that range from 2–20 depending on the reference radiation, biological end point, and cell type (26–30). Among them are the RBEs at D_{37} for ^{241}Am α particles, compared to acute ^{137}Cs γ rays, of 7.6 and 12 for AG1522 cells (31) and DU-145 cells (32), respectively.

Table 4 indicates that the experimental D_{37} depends on the percentage of cells in the cluster that were radiolabeled. Mean lethal doses of 0.64, 0.76 and 1.1 Gy were obtained for 100, 10 and 1% labeling, respectively. Similar differences in the D_{37} were seen (Table 4) when the cells were labeled with ^{131}IdU (11). Therefore, the mean cluster dose alone cannot be used to predict the first two logs of killing caused by either ^{210}Po -citrate or ^{131}IdU . However, in the case of ^{131}IdU , semi-empirical modeling of the response provided excellent fits to the first two logs of cell killing for each labeling condition [Fig. 1 of ref. (24)]. In the present work, a more detailed model was implemented for the ^{210}Po -citrate. Based on Fig. 6 and the P values, this approach reasonably predicts the slope of the first two logs of cell killing for 100 and 10% labeling but not 1% labeling.

The experimental design in the present study is reminiscent of studies on the radiobiology of hot particles (33–35). The D_{37} results in Table 4 indicate that, for the same mean absorbed dose to the cluster, cell killing increases with increasing percentage of labeled cells (within the first two logs). That is, the biological effect of the radioactivity increases as the distribution of radioactivity becomes more uniform. This was observed for both ^{210}Po (α particles) and ^{131}IdU (β particles). These findings for cell survival are similar to those found for carcinogenesis end points when liver, lung and skin were exposed to different concentrations of hot particles (33–35). For estimation of carcinogenic risk from hot particles, use of the mean absorbed dose to the organ or tissue will result in the most conservative estimate of safety, and thus it should be used. However, for targeted radionuclide therapy of cancer, use of the mean absorbed dose to predict cell killing caused by nonuniform distributions of radioactivity can exaggerate the response and consequently the potential therapeutic benefit (36,37).

Although the first two logs of cell killing follow the exponential pattern anticipated for α particles, Figs. 5 and 6 show that the response begins to saturate when the SF drops below ~1% ($P = 0$). This occurs for all three labeling conditions. Most surprising is 100% labeling, where cross-irradiation would be expected to provide a fairly uniform dose distribution across all cells in the cluster. No saturation was observed when 100% of the cells were labeled with ^{131}IdU ,

which emits long-range β particles (11). However, saturation was observed when 10% of the V79 cells in the multicellular cluster were labeled with ^{131}IdU . Our studies with lethally irradiated feeder cells described above indicate that feeder effects (17) are not present in our cultures and therefore do not appear to play a role in this phenomenon. Furthermore, when the multicellular clusters are irradiated uniformly either chronically or acutely with ^{137}Cs γ rays, no saturation in the survival curves was observed (Fig. 5). Hence the saturation of the survival curves observed at high doses does not appear to be an artifact.

To investigate potential causes of the survival curve saturation, we assessed the distribution of radioactivity among the cells that were labeled with ^{210}Po -citrate (16). These studies showed that there is a log-normal distribution of ^{210}Po -citrate among the labeled cells (16). The log-normal shape of the activity distribution remained very similar over a range of extracellular concentrations (0–67 kBq/ml). The shape was essentially the same before and 24 h after plating the cells for *CF* (16). The nonuniform activity distribution at the cellular level invariably leads to nonuniform dose distributions. Theoretical calculations showed that for *isolated* cells (i.e. no cross-dose) labeled with ^{210}Po -citrate, the survival curves are expected to saturate when the activity distribution is log-normal (16). In the multicellular cluster, the lognormal distribution of cellular activity will certainly lead to log-normal self-dose distributions and will also have an impact on the breadth of the cross-dose distribution. One can anticipate that as the percentage of labeled cells decreases, the mean cross-dose will increasingly overestimate the cross-doses received by the unlabeled cells far from labeled cells. Correspondingly, the cross-doses to nearby cells will be higher than the mean cross-dose. The fact that our semi-empirical model (Eq. 10) uses mean self- and cross-doses may explain in part why the model fails to predict the saturation in the survival curves. It should be noted that the cross-dose distribution also depends on other geometrical considerations such as packing density, variation in cellular dimensions, type of radiation, and energy emitted (3,5,6,22,38). Consideration of these aspects in the context of log-normal activity distributions may help us understand and model the complete dose–response curves observed in the present work. Finally, additional geometrical considerations related to the shape of the multicellular clusters or perhaps to a biological phenomenon such as bystander effects or adaptive responses may also be involved (39,40). These possibilities are under investigation.

Last, it is of interest to examine to what extent the relatively long physical half-life (T_p) of ^{210}Po (138.4 days) affects our findings and their extrapolation to other α -particle emitters. Most α -particle emitters that have been proposed for use in therapeutic nuclear medicine have much shorter T_p (~1 h to ~10 days). The labeled cells in the present experiments receive doses during the *I*, *M* and *CF* periods. The relative contributions of the doses delivered to the cells during these periods depend on T_p . Therefore, if the dose response of the cells were different during these periods, T_p could have some impact on the shape of the resulting survival curves. Arguing against this is the similarity between the D_{37} value of 0.64 Gy obtained for 100% labeling (Table 4), where most of the dose is delivered during the maintenance period, and the value of 0.68 Gy that was obtained for isolated cells where essentially all of the dose was delivered during the *CF* (23). Therefore, similar findings should be anticipated for α -particle emitters with somewhat shorter physical half-lives. However, other aspects related to the dependence of RBE on emitted α -particle energies and chord-length distributions would have some impact on the survival curves (41,42).

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FIG. 1.

Representative temporal dependence of intracellular activity of ^{210}Po -citrate in the labeled cells for the 100% labeling case where 1 mBq/cell is taken up by the V79 cells by the end of the uptake period. The area under the curve is proportional to the cumulated decays in the V79 cell. The inset shows the corresponding cumulated activity as a function of time.

FIG. 2.

Dependence of cellular uptake of ^{210}Po on initial extracellular concentration of ^{210}Po -citrate in the culture medium. Different symbols correspond to data collected in different independent experiments in the cases of 100% (left), 10% (center) and 1% (right) labeling conditions. Thin solid and dashed lines represent linear least-squares fits to the data for individual experiments. Thick solid lines represent fits to the composite data.

FIG. 3.

Survival of V79 cells in multicellular clusters as a function of initial ^{210}Po activity per labeled cell when 100, 10 or 1% of the cells in the multicellular clusters were labeled with ^{210}Po -citrate. In each graph, different symbols correspond to independent experiments. Solid curves represent least-squares fits of the data by a two-component exponential function.

FIG. 4. Survival of V79 cells in multicellular clusters as a function of initial ^{210}Po activity per cluster (in kBq). Least-squares fits of the data by a two-component exponential function are shown for each labeling condition: 100% (solid line), 10% (dashed line), and 1% (dot-dashed line).

FIG. 5. Comparison of dose-response curves for external chronic ^{137}Cs γ rays (dotted), ^{131}IdU (10% labeled, long-dashed), and ^{210}Po -citrate (100% labeled, solid; 10% labeled, medium-dashed; 1% labeled, dot-dashed). The abscissa represents the mean absorbed dose to the cluster.

FIG. 6.

SF of a mixed population of unlabeled cells and cells labeled with ^{210}Po -citrate in multicellular clusters. The mean self-dose to the labeled cells, $D_{\text{self}}^{\text{labeled}}$, is given on the lower abscissa. The mean cross-dose to the unlabeled cells, $D_{\text{cross}}^{\text{unlabeled}}$, is given on the upper abscissa. The solid lines represent the SF of the mixed population of labeled and unlabeled cells predicted by Eq. (10).

TABLE 1
Parameters used in Preparation of Multicellular Clusters

Cells labeled with ^{210}Po -citrate (%)	Activity concentrations for cell labeling κ (kBq/ml)	Incorporated radioactivity per labeled cell A_0 (mBq/cell)	Number of labeled cells per cluster ^a
Control	0	0	0
100	0–9	0–0.1	4×10^6
10	0–36	0–1	4×10^5
1	0–160	0–40	4×10^4

^aTotal number of labeled and unlabeled cells in the cluster is 4×10^6 .

TABLE 2
Cellular Uptake of ^{210}Po as a Function of Extracellular Concentration of ^{210}Po -citrate

Percentage labeled cells and experiment no.	Slope	210Po-citrate recipe and average slope		
	mBq/cell	Flask passage no.	210Po-citrate age (days)	mBq/cell
	kBq/ml			kBq/ml
100%				
1	0.015 ± 0.001	5	3	(PoCl4;Na-citrate):MEMB (1:7):19 0.028 ± 0.002
2	0.008 ± 0.001	8	14	
10%				
1	0.028 ± 0.004	14	1	(PoCl4;Na-citrate):MEMB (1:7):1 0.116 ± 0.026
2	0.024 ± 0.004	9	1	
3	0.027 ± 0.004	19	0	
1%				
1	0.180 ± 0.034	3	5	(PoCl4;Na-citrate):MEMB (1:7):1 0.116 ± 0.026
2	0.241 ± 0.026	8	2	
3	0.022 ± 0.004	11	13	
4	0.008 ± 0.004	20	0	

TABLE 3

Fitted Parameters for Multicellular Cluster Survival Curves

Cells labeled with ^{210}Po -citrate (%)	b	A_1^a (mBq/cell)	A_2^a (mBq/cell)	A_1^b (kBq/cluster)	A_2^b (kBq/cluster)	R^2
100	0.012 ± 0.001	0.005 ± 0.001	0.030 ± 0.006	0.021 ± 0.003	0.120 ± 0.026	0.92
10	0.024 ± 0.005	0.068 ± 0.005	0.619 ± 0.440	0.027 ± 0.002	0.247 ± 0.176	0.94
1	0.021 ± 0.004	0.999 ± 0.078	5.033 ± 1.280	0.040 ± 0.003	0.201 ± 0.051	0.93

^aLeast-squares fit to data in Fig. 3.

^bLeast-squares fit to data in Fig. 4.

TABLE 4
Mean Lethal Doses and RBE Values for V79 Multicellular Clusters

Treatment	Cells labeled (%)	D_{37} (Gy)	RBE
^{137}Cs γ rays ^a (chronic)	—	12.20 ± 2.41	1
^{210}Po -citrate	100	0.64 ± 0.10	19.1 ± 4.8
^{210}Po -citrate	10	0.76 ± 0.06	—
^{210}Po -citrate	1	1.13 ± 0.09	—
$^{131}\text{IdU}^a$	100	4.51 ± 0.18	2.7 ± 0.5
$^{131}\text{IdU}^a$	10	5.72 ± 0.66	—
$^{131}\text{IdU}^a$	1	6.36 ± 0.48	—

^aData from ref. (11), provided for comparison.

TABLE 5
Mean Cellular Self- and Cross-Doses when 100, 10 or 1% of the Cells are Labeled^a

Period	$\frac{\tilde{A}_{\text{source}}}{A_0} \text{ period } b$ (s)	Type, target←source	S(targets←source)	100% D_{labeled} (Gy/mBq)	10% D_{labeled} (Gy/mBq)	1% D_{labeled} (Gy/mBq)	10% $D_{\text{unlabeled}}$ (Gy/mBq)	1% $D_{\text{unlabeled}}$ (Gy/mBq)
Uptake (<i>t</i>)	9.0×10^2	Self, cell←cell	9.5×10^{-2}	0.1	0.1	0.1	0	0
Maintenance (<i>M</i>)	1.8×10^3	Cross, medium←medium	4.3×10^{-10}	0.1	0.1	~0	0	0
	2.6×10^5	Self, cell←cell	9.5×10^{-2}	24.3	24.3	24.3	0	0
Colony formation (<i>CF</i>)	2.6×10^5	Cross, cell←cluster	1.1×10^{-7}	112.8	11.3	1.1	11.3	1.1
	7.0×10^4	Self, cell←cell	9.5×10^{-2}	6.6	6.6	6.6	0	0
Total dose	2.8×10^5	Cross, cell←adjacent cell	1.1×10^{-2}	2.9	2.9	2.9	0	0
		Self		31.0	31.0	31.0	0	0
		Cross		115.8	14.2	4.0	11.3	1.1

^a Absorbed doses are given in Gy per mBq per labeled cell.

^b This quantity is often referred to as the residence time (τ).