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Modeling Multicellular Response to Nonuniform Distributions of Radioactivity: Differences in Cellular Response to Self-Dose and Cross-Dose

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Abstract

Radiopharmaceuticals are distributed nonuniformly in tissue. While distributions of radioactivity often appear uniform at the organ level, in fact, microscopic examination reveals that only a fraction of the cells in tissue are labeled. Labeled cells and unlabeled cells often receive different absorbed doses depending on the extent of the nonuniformity and the characteristics of the emitted radiations. The labeled cells receive an absorbed dose from radioactivity within the cell (self-dose) as well as an absorbed dose from radioactivity in surrounding labeled cells (cross-dose). Unlabeled cells receive only a crossdose. In recent communications, a multicellular cluster model was used to investigate the lethality of microscopic nonuniform distributions of ¹³¹I iododeoxyuridine (¹³¹IdU). For a given mean absorbed dose to the tissue, the dose response depended on the percentage of cells that were labeled. Specifically, when 1, 10 and 100% of the cells were labeled, a D_{37} of 6.4, 5.7 and 4.5 Gy, respectively, was observed. The reason for these differences was recently traced to differences in the cellular response to the self- and cross-doses delivered by ¹³¹IdU. Systematic isolation of the effects of self-dose resulted in a D_{37} of 1.2 ± 0.3 Gy. The cross-dose component yielded a D_{37} of 6.4 ± 0.5 Gy. In the present work, the overall survival of multicellular clusters containing 1, 10 and 100% labeled cells is modeled using a semi-empirical approach that uses the mean lethal self- and cross-doses and the fraction of cells labeled. There is excellent agreement between the theoretical model and the experimental data when the surviving fraction is greater than 1%. Therefore, when the distribution of ¹³¹I in tissue is nonuniform at the microscopic level, and the cellular response to self- and cross-doses differs, multicellular dosimetry can be used successfully to predict biological response, whereas the mean absorbed dose fails in this regard.

INTRODUCTION

Radioactivity can enter the body through a variety of pathways; among them are inhalation, ingestion, absorption through skin, and injection (vein, artery, peritoneum, intrathecal, etc.). In general, regardless of the pathway, the radioactivity distributes nonuniformly in the organs and tissues within the body. The nonuniformities in the distribution occur over the entire range of spatial dimensions relevant to the body. That is, there is differential uptake of the radioactivity among the organs in the body, among the numerous tissues within an organ, and even among like cells that constitute a small tissue element within an organ (1–3). The variability in uptake of radioactivity among like cells within a tissue element can be very large. Often, some cells may contain no radioactivity while the others have widely differing amounts

of radioactivity. These concepts are reviewed in detail in a recent report of the International Commission on Radiation Units and Measurements (ICRU) (4,5).

The inherent nonuniform distribution of radionuclides in tissues has made it exceedingly difficult to predict the biological effects caused by their emitted radiations. Depending on the degree of nonuniformity of the distribution of radioactivity and the range of the emitted radiations, the cellular absorbed doses can vary by several orders of magnitude (3,6–9). As a consequence, the biological response of the cells can vary similarly (10–13). Complicating this problem is the fact that the relative biological effectiveness (RBE) of the radiations emitted by decays within the cell that deposit self-dose can be quite different from the RBE from radiations emitted by decays in neighboring cells that deposit cross-dose (9,14). In these instances, the mean absorbed dose does not adequately predict biological response (4,5). However, despite its obvious shortcomings, the mean absorbed dose to the organ or tissue is still generally used to predict response for therapeutic exposures and risk from low-level exposures (15).

In an earlier study, the radiotoxicity of nonuniform distributions of ^{131}I -iododeoxyuridine (^{131}IdU) was examined using an experimental multicellular cluster model wherein 1, 10 or 100% of the cells in the cluster were labeled (16). The principal β particles emitted by ^{131}I have a mean energy of 191 keV and a mean range in water of about 400 μm . Therefore, when this radionuclide is localized within a cell, the β particles emitted typically cross-irradiate target cells within 30–40 cell diameters. Despite the capacity of these β particles to irradiate neighboring cells, it was demonstrated that the mean absorbed dose did not adequately predict the response of the cell population in the multicellular cluster (16). The reasons for this were primarily the different cellular response to the self-dose and cross-dose and the nonuniform distribution of radioactivity (16). In the present work, a multicellular dosimetry approach is used to develop a theoretical model to predict the biological response of tissues to nonuniform distributions of radioactivity. This model differs from other approaches in that it explicitly accounts for differences in cellular response to self- and cross-dose. The model is tested against the aforementioned experimental data on nonuniform distributions of ^{131}IdU in multicellular clusters (16).

METHODS

When radioactivity is distributed nonuniformly in tissue, the labeled cells receive a self-dose (D_{self}) from radiations emitted by radionuclide decays within that cell. The labeled cells also receive a cross-dose (D_{cross}) from radionuclide decays in other labeled cells in the tissue (8, 9,17,18). Unlabeled cells receive only the cross-dose (D_{cross}). Therefore, the biological response of the labeled cells in the tissue can be very different from the response of the unlabeled cells. This was demonstrated recently by Neti and Howell using a multicellular cluster containing 50% cells labeled with ^{131}IdU and cell survival as the biological end point (14). Having succeeded in measuring the individual responses to the self- and cross-doses, one can develop a theoretical dose–response model for a mixed population of labeled and unlabeled cells in the following manner. If f represents the fraction of cells that are labeled and $(1 - f)$ the fraction of cells that are unlabeled, then the survival of the mixed population of cells SF_{mixed} can be written as

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

where $\text{SF}_{\text{labeled}}$ and $\text{SF}_{\text{unlabeled}}$ are the fraction of labeled cells that survive and the fraction of unlabeled cells that survive, respectively. The definitions of these and other symbols are summarized in Table 1 for convenience. In principle, any appropriate response function can

be used for SF_{labeled} and $SF_{\text{unlabeled}}$ (19). For ^{131}IdU (14), it has been shown that the response of the unlabeled cells can be described by a simple exponential function

$$SF_{\text{unlabeled}} = e^{-D_{\text{cross}}/D_{37,\text{cross}}}, \quad (2)$$

where $D_{37,\text{cross}}$ is the cross-dose required to achieve 37% survival. The radiation response of the labeled cells arises from both the self-dose and the cross-dose. For ^{131}IdU (14), the labeled cells have been shown to respond according to the product of two exponential functions,

$$SF_{\text{labeled}} = e^{-D_{\text{self}}/D_{37,\text{self}}} e^{-D_{\text{cross}}/D_{37,\text{cross}}}, \quad (3)$$

where $D_{37,\text{self}}$ is the self-dose required to achieve 37% survival. The cellular self-dose can be calculated using the methods and tables contained in refs. (20,21). As noted earlier, the cellular response to self-dose can be quite different from the cross-dose. For ^{131}IdU , the self-dose has been shown to be substantially more lethal per unit dose than the cross-dose (14). Even larger differences can be anticipated for Auger electron emitters such as ^{125}I (22,23) and ^{195}mPt (24), which have very high cellular self-dose RBE values. Substitution of Eqs. (2) and (3) into Eq. (1) and simplifying yields

$$SF_{\text{mixed}} = e^{-D_{\text{cross}}/D_{37,\text{cross}}} \left[f e^{-D_{\text{self}}/D_{37,\text{self}}} + (1 - f) \right]. \quad (4)$$

This expression explicitly accounts for differences in doses received by the labeled and unlabeled cells (D_{self} , D_{cross}), differences in the biological response to self-dose and cross-dose ($D_{37,\text{self}}$, $D_{37,\text{cross}}$), and the fraction of cells labeled (f). As described by Goddu *et al.* (9), it can be convenient to express the response in terms of the ratio R of the cellular self-dose to cross-dose (9).

$$R = D_{\text{self}}/D_{\text{cross}}. \quad (5)$$

This quantity is closely related to the geometric enhancement factor of Humm and Cobb (8) and the cellular to conventional dose ratio of Makrigiorgos *et al.* (7). Solving Eq. (5) for D_{self} and substituting into Eq. (4) yields

$$SF_{\text{mixed}} = e^{-D_{\text{cross}}/D_{37,\text{cross}}} \left[f e^{-RD_{\text{cross}}/D_{37,\text{self}}} + (1 - f) \right]. \quad (6)$$

Alternatively, solving Eq. (5) for D_{cross} and substituting into Eq. (4) yields

$$SF_{\text{mixed}} = e^{-D_{\text{self}}/RD_{37,\text{cross}}} \left[f e^{-D_{\text{self}}/D_{37,\text{self}}} + (1 - f) \right]. \quad (7)$$

These expressions are particularly useful when the cross- or self-dose is known and the ratio of self-dose to cross-dose can be estimated from a mathematical model of the tissue (9).

The capacity of the multicellular dosimetry-based model described by Eqs. (4), (6) and (7) to predict biological response can be tested using the experimental data published in our earlier reports (14,16). Response curves were obtained for multicellular clusters containing different percentages (100, 10 and 1%) of Chinese hamster V79 cells labeled with ^{131}IdU (16). Cell survival was plotted as a function of mean activity per labeled cell, cluster activity, and mean

absorbed dose to the cluster. It was apparent that none of these were useful quantities for predicting the response of the mixed population of labeled and unlabeled cells. Accordingly, further work was undertaken to examine the individual responses of the labeled and unlabeled cells using a fluorescence-activated cell sorter (FACS) (14). These efforts led to experimentally measured values of $D_{37,\text{self}}$ and $D_{37,\text{cross}}$ for the multicellular clusters of cells. The values were 1.2 and 4.0 Gy, respectively, clearly indicating that there is a substantial difference between the lethality of the self- and cross-doses from ^{131}IdU (14). While the value for $D_{37,\text{self}}$ was consistent with related experimental data for ^{131}IdU (25,26), the value for $D_{37,\text{cross}}$ was not (14). Studies in which 1% of the cells were labeled with ^{131}IdU yielded a value for $D_{37,\text{cross}}$ of 6.4 Gy. This value was used in the present modeling since it is considered to have a higher degree of accuracy (14,16). The reasons for this are complex and are outside the scope of this article. A detailed discussion can be found in refs. (14,16). These data, which are used in the theoretical model, are summarized in Table 2.

RESULTS

Using the experimental multicellular cluster data on the surviving fraction of the mixed populations of cells (labeled and unlabeled) as a function of ^{131}I activity per labeled cell [Fig. 3 in ref. (16)], and the values of D_{self} and D_{cross} in Table 2, the surviving fraction is replotted in Fig. 1. The bottom and top abscissas are the average self-dose to the labeled cells and the average cross-dose to the labeled and unlabeled cells, respectively. Note that the ratios of self-dose to cross-dose vary by 10-fold between each successive labeling condition (100%, 10%, 1%), with the ratio for 10% labeling being close to unity (Table 2). The solid lines represent the response modeled by the function in Eq. (6). The model parameters $D_{37,\text{self}}$ and $D_{37,\text{cross}}$ were fixed at 1.2 Gy and 6.4 Gy, respectively, while the parameters R and f were changed depending on the fraction of cells labeled (Table 2).

To assess how well the theoretical model fits the experimental data, the Fit Comparison tool in Origin (Originlab Corp., Northampton, MA) was used. With this tool, the experimental data were compared to data predicted by the theoretical model by fitting the respective data sets to a simple two-component exponential function that was used previously to fit the experimental data (16). Origin then combined the experimental and theoretical data sets and fitted the composite data to the same two-component exponential function. The sum of squares was then calculated by Origin for each data set relative to the composite fit values. In addition, the number of degrees of freedom was calculated for each case. The Fit Comparison tool then implemented an F test. Based on the results of the F test, a P value was generated by Origin and used to ascertain whether the data in question were significantly different from the composite data. A P value <0.05 indicates that the data are significantly different. When $P = 1.0$, the data are essentially a match. Table 3 gives the P values for the experimental data and theoretical model. There is excellent agreement between the experimental data and theoretical model when 100% and 1% of the cells are labeled. In the case of 10% labeling, the correspondence between the theoretical model and the experimental data is poor ($P = 0$; Table 3, column 4, row 2). However, in Fig. 1B the model predictions appear to be excellent above 1% survival. Repeating the fit comparison on these theoretical data ($0.01 < \text{SF} \leq 1.0$) for 10% labeling yields a P value of 0.99. A P value of 0.99 was also obtained for the experimental data. In summary, the above statistical results indicate that, for all labeling conditions, the surviving fractions predicted by our model are very close to the experimental data when the surviving fractions are greater than 1%. In the cases of 100% and 1% labeling, the theoretical model adequately predicts the response over the entire range of survivals for which experimental data were obtained.

To more broadly examine the dependence of biological response on the fraction of cells labeled in a multicellular cluster, predicted survival curves based on the model represented by Eq. (6)

are shown in Fig. 2 for the cases of 100, 90, 70, 50, 10 and 1% labeling. The self-dose to cross-dose ratios used in these calculations were 0.121, 0.134, 0.173, 0.242, 1.21 and 12.1, respectively. As explained earlier, the values for $D_{37,\text{self}}$ and $D_{37,\text{cross}}$ were fixed at 1.2 Gy and 6.4 Gy, respectively (Table 2).

DISCUSSION

When predicting the radiobiological effects of radioactivity in tissue, it is common practice to assume that the radioactivity is distributed uniformly in the tissue and that all of the tissue receives the mean absorbed dose. However, it was clearly shown previously that the mean absorbed dose to the tissue as a whole cannot be used reliably to predict the lethality of ^{131}IdU when the radioactivity was distributed uniformly at the macroscopic level but nonuniformly at the microscopic (multicellular) level (16). This was true at both low and high doses (16). This result was somewhat surprising in view of the capacity of the medium-energy β particles emitted by ^{131}I to effectively cross-irradiate unlabeled cells due to their relatively long range in tissue (average range of 30–40 cell diameters) (16).

The reasons for the inability of the mean absorbed dose to predict response to the radiation from ^{131}IdU appear to be manifold. First and foremost is the difference in lethality between the self-dose from radioactivity within the cell and the cross-dose from radioactivity in surrounding cells. Neti and Howell (14) showed that the cellular self-dose from ^{131}IdU is substantially more radiotoxic than the cross-dose. The impact of this RBE on the overall response of the mixed population of labeled and unlabeled cells depends on the fraction of cells that are labeled (14). Accordingly, the multicellular dosimetry approach used in this work takes into account the mean self-dose to the labeled cells, the mean cross-dose to the labeled and unlabeled cells, the fraction of cells labeled, dose response to self-dose, and dose response to cross-dose. As shown in Fig. 1, unlike approaches that use the mean absorbed dose to the cluster, this multicellular dosimetry approach does an excellent job of predicting the lethality of nonuniform distributions of ^{131}IdU down to about 1% survival. This is a significant improvement over approaches that simply use the mean absorbed dose to the tissue (14).

The multicellular dosimetry-based model has also been used to broadly examine the impact of nonuniform distribution of radioactivity on the lethality of ^{131}IdU . The results in Fig. 2 show that small reductions in the percentage of cells labeled can have a substantial impact on the biological response. Notable changes are observed as the percentage changes from 100% to 90, 70, 50 and 10%. The contribution of the self-dose to the killing of the mixed population of cells diminishes as the percentage of labeled cells drops. The self-dose plays a substantial role in the overall response when high percentages of the cells are labeled even though the self-dose is only a fraction of the cross-dose (Table 2). This is because the lethality of the self-dose is more than five times that of the cross-dose (Table 2, columns 5 and 6). As the percentage of labeled cells drops, the self-dose plays an increasingly important role in killing labeled cells because of the increase in the ratio of self-dose to crossdose and increased activity per cell required to achieve the same level of killing of the mixed cell population. However, death of labeled cells becomes inconsequential to the overall response because the labeled cells constitute a decreasing fraction of the mixed population. At 10% labeling, the overall response is already dominated by unlabeled cells, which receive only cross-dose. Accordingly, little change in the survival curves for the mixed population is apparent as the percentage drops further from 10% to 1% (Fig. 2). Thus the central message of Fig. 2 is that the percentage of cells that are labeled has a profound impact on the response for this medium-energy β -particle emitter and that a multicellular dosimetry approach can be used to predict the overall response.

Although the multicellular dosimetry approach used in this work does an excellent job of predicting the lethality of nonuniform distributions of ^{131}I down to about 1% survival, Fig. 1B

shows that the model fails for surviving fractions below 1% ($P = 0$ in Table 3, column 4, row 2). This was discussed by Neti and Howell (16), who suggested that it may be due to geometrical considerations in the experimental multicellular cluster model or perhaps some biological phenomenon. If geometrical considerations are the cause, methods that employ Monte Carlo techniques (11) or microdosimetric moments (12) may need to be employed. This is presently under investigation. It should also be pointed out that the current theoretical model uses mean self- and cross-doses. Variations of activity per labeled cell may also play a role in determining the dose–response curve beyond 1% survival.

Finally, it should be noted that this theoretical model is likely to be applicable under certain circumstances for classes of radionuclides other than energetic β -particle emitters. This is because the radiotoxicity of the self- and cross-doses are accounted for individually, a feature required by many Auger electron-emitting radionuclides (e.g. ^{111}In , ^{125}I). However, caution should be exercised, particularly when very small fractions of cells are labeled. In these instances, when relatively short-range radiations are responsible for the cross-dose, there can be wide variation in the cross-dose received by the cells. This may lead to a highly variable response in the unlabeled cell population. The present theoretical model may not be adequate in this situation. However, multicellular dosimetry approaches can be designed to handle such cases, as well as biological phenomena such as bystander effects (27–31).

CONCLUSIONS

When the distributions of ^{131}I in tissue is nonuniform at the microscopic level, multicellular dosimetry can be used successfully to predict biological response, whereas the mean absorbed dose fails in this regard.

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FIG. 1.

Survival of a mixed population of unlabeled cells and cells labeled with ^{131}IdU in multicellular clusters. The mean cellular self-dose to the labeled cells is given on the lower horizontal axis. The mean cross-dose to the labeled and unlabeled cells is given on the upper horizontal axis. The data plotted in the three panels are from experiments in which different percentages of cells in the multicellular cluster were labeled with ^{131}IdU (16). Panel A: 100% ($\circ$); panel B: 10% ($\square$); panel C: 1% ($\triangle$). The inset in panel A is an enlargement of the low-dose region. The clusters were maintained at 10.5°C for 72 h and then the surviving fractions were determined compared to cells from control clusters (0% labeled). Data from three independent experiments

are plotted for each labeling condition. The solid lines represent the surviving fraction of the mixed population predicted by the model $SF_{\text{mixed}} = e^{-D_{\text{cross}}/D_{37,\text{cross}}} [f e^{-R D_{\text{cross}}/D_{37,\text{self}}} + (1 - f)]$ where f is the fraction of cells labeled, D_{cross} is the cross-dose received by the labeled and unlabeled cells, and R is the ratio of self-dose to cross-dose for the labeled cells. The parameters that describe cellular response to the self- and cross-doses, $D_{37,\text{self}}$ and $D_{37,\text{cross}}$, are fixed regardless of the percentage cells labeled. These model parameters are given in Table 2.

FIG. 2.

Predicted survival curves for three-dimensional multicellular clusters containing different mixtures of unlabeled cells and cells containing $^{131}\text{IIdU}$. The surviving fraction, calculated using Eq. (6) and the parameters in Table 2, is plotted as a function of the mean cross-dose to the cells in the cluster.

TABLE 1

Definition of Symbols

Symbol	Definition
SF_{labeled}	Surviving fraction for cells labeled with radioactivity
$SF_{\text{unlabeled}}$	Surviving fraction for unlabeled cells (not labeled with radioactivity)
SF_{mixed}	Surviving fraction for a mixed population of labeled and unlabeled cells
f	Fraction of cells in the mixed population that are labeled with radioactivity
D_{self}	Absorbed dose to the target cell from radiations emitted by decays within the target cell
D_{cross}	Absorbed dose to the target cell from radiations emitted by decays outside the target cell
$D_{37,\text{self}}$	Self-dose required to achieve 37% survival
$D_{37,\text{cross}}$	Cross-dose required to achieve 37% survival
R	Ratio of self-dose to cross-dose ($D_{\text{self}}/D_{\text{cross}}$)

TABLE 2

Summary of Dosimetry Quantities Used for Modeling

Percentage cells labeled	D_{self} (Gy per mBq) ^{a,b}	D_{cross} (Gy per mBq) ^{a,b}	$R = D_{\text{self}}/D_{\text{cross}}$	$D_{37,\text{self}}$ (Gy) ^b	$D_{37,\text{cross}}$ (Gy) ^c
100	0.32	2.64	0.121	1.2	6.4
90	0.32	2.39	0.134	1.2	6.4
70	0.32	1.85	0.173	1.2	6.4
50	0.32	1.32	0.242	1.2	6.4
10	0.32	0.264	1.21	1.2	6.4
1	0.32	0.0264	12.1	1.2	6.4

^aGy per mBq per labeled cell.

^bTaken from ref. (14).

^cTaken from ref. (16).

TABLE 3

Comparison of Statistical Analyses of Experimental Data and Theoretical Model

Percentage labeled cells	Range of experimental data considered	Experimental <i>P</i> value	Model <i>P</i> value
100%	all	0.95	0.95
10%	all	0.91	0
10%	$0.01 < SF \leq 1.0$	0.99	0.99
1%	all	1.0	1.0