Published in final edited form as:

Int J Radiat Biol. 2012 March ; 88(3): 286–293. doi:10.3109/09553002.2012.638357.

Flow cytometry-assisted Monte Carlo simulation predicts clonogenic survival of cell populations with lognormal distributions of radiopharmaceuticals and anticancer drugs

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Abstract

Purpose—Although the distribution of therapeutic agents within cell populations may appear uniform at the macroscopic level, the distribution at the multicellular level is nonuniform. As such, the mean agent concentration in tissue may not be a suitable quantity for use in predicting biological effects. Failure in chemotherapy and targeted radionuclide therapy has been attributed, in part, to the ubiquity of lognormal distributions of therapeutic agents. To improve capacity to predict biological response, this work develops approaches that determine the fate of a cell population on a cell-by-cell basis.

Methods—Incorporation of the α -particle emitting radiochemical (^{210}Po -citrate) and two anticancer drugs (daunomycin and doxorubicin) by Chinese hamster V79 cells was determined using flow cytometry. Monte Carlo simulation was used to estimate cell survival on the bases of mean and individual cell incorporation of each cytotoxic agent. The interrelationships between the Monte Carlo simulated cell survival and clonogenic cell survival were evaluated.

Results—Cell survival obtained by Monte Carlo simulation based on individual cell incorporation was in good agreement with clonogenic cell survival for all agents. However, the agreement was poor when the simulation was carried out using the mean cell incorporation of the agents.

Conclusion—These data indicate that, with the aid of flow cytometry, Monte Carlo simulations can be used to predict the toxicity of therapeutic agents in a manner that takes into account the effects of lognormal and other nonuniform distributions of agents within cell populations.

Keywords

Alpha emitters; chemotherapy; clonogenic cell survival; radionuclides; prediction of tumour response; chemo/radiotherapy

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

Introduction

A significant effort has been directed toward achieving improvements in treatment of cancer with radioimmunotherapy (Michel et al. 2005, Sharkey and Goldenberg 2008, Shen et al. 2010, Song and Sgouros 2011), radiolabeled chemotherapy agents (Hou et al. 1985, Azure et al. 1992, Howell et al. 1994, Ickenstein et al. 2006), and a combination of chemotherapy with radioimmunotherapy (DeNardo et al. 1997, Costantini et al. 2010). While considerable successes have been realized with targeted radionuclide therapy and translated into frontline therapies (Song and Sgouros 2011), the full potential of this therapeutic modality has yet to be realized. Unfavorable outcomes in targeted radionuclide therapy and chemotherapy may be attributable, in part, to the fact that the distributions of these agents in tissue are not only nonuniform, but are also lognormal (Neti and Howell 2006, 2008, Akudugu et al. 2011). As such, some malignant cells may take up very little or no therapeutic agent, leading to minimal or no toxic effects on such subpopulations depending on the extent of cross-irradiation from nearby cells that have taken up the radiopharmaceutical (Goddu et al. 1994, Rajon et al. 2011). It is likely that tumor repopulation by under-targeted subpopulations of cells in either the primary tumor or in metastases leads to therapeutic failure.

The therapeutic significance of nonuniform incorporation of chemotherapy drugs and radiopharmaceuticals by cancer cells has been emphasized by many studies (Rossi and Ellis 1952, Durand 1981, Wessels and Rogus 1984, Humm 1986, Howell et al. 1989, Luk and Tannock 1989, Makrigiorgos et al. 1990, Goddu et al. 1994, O'Donoghue 1999, Pinto and Howell 2007, Aroui et al. 2010). Yet, with the exception of a few reports (Neti and Howell 2006, 2008, Akudugu et al. 2011, Rajon et al. 2011), the impact of lognormal drug distributions on the capacity to sterilize a population of cells appears to be largely unexplored. A small lognormal shape parameter (σ) implies a narrow distribution profile and $\sigma \rightarrow 0$ when all cells incorporate the same amount of agent. On the other hand, a large σ signifies a wide spread in distribution and agent incorporation may range from very low (potentially non-toxic) to high (very lethal). A recent study provided some evidence of the ubiquity of lognormal drug distributions by using flow cytometry to assess the distribution of ^{210}Po -citrate, daunomycin, and doxorubicin (Akudugu et al. 2011). Equally important was the demonstration that changes in the value of σ as a function of increasing drug concentration paralleled marked changes in the shapes of the corresponding clonogenic cell survival curves (Akudugu et al. 2011). Unanswered, however, was whether the experimental lognormal distributions (i.e., individual drug uptake on a cell-by-cell basis) could be used to accurately predict the saturation that was observed in the experimental cell survival-curves (e.g., two-component exponential). This saturation has been observed repeatedly in our studies on the lethal effects of nonuniform distributions of radioactivity (Neti and Howell 2003, 2004, 2006, 2007, 2008). Furthermore, theoretical studies show that lognormal distributions can lead to such two-component exponential survival curves in both monolayer and three-dimensional tissue constructs (Neti and Howell 2006, Rajon et al. 2011).

The present study describes how clonogenic cell survival can be predicted based only on knowledge of the initial slope of the cell survival curve and information on the distribution of agent incorporation among the treated cell population. To achieve this, the distribution of ^{210}Po , daunomycin and doxorubicin among populations of Chinese hamster V79 cells

was assessed using flow cytometry techniques and used to theoretically model the surviving fraction. Daunomycin and doxorubicin were selected because they are chemotherapy drugs with natural fluorescence properties. ^{210}Po was selected as a surrogate for α -emitting radionuclides used in radioimmunotherapy. Two modeling approaches were compared, including Monte Carlo simulation of cell survival based on the experimental distributions of drug uptake, and Monte Carlo simulation of cell survival based on the more conventional approach of using mean cellular uptake of the drugs. These studies were undertaken to shed light on how cell-specific analysis of drug uptake and survival can be used to predict the response of cell populations to cytotoxic drugs and targeted radionuclide therapies.

Materials and methods

Experimental cellular uptake and toxicity of agents

The agent uptake profiles and toxicity of ^{210}Po -citrate, daunomycin, and doxorubicin in Chinese hamster V79 lung fibroblasts were described in detail previously (Akudugu et al. 2011). Briefly, after treatment of the cells with each agent, uptake of the agent by each cell of the population was quantified by flow cytometry. Both daunomycin (Sigma, cat. # D8809) and doxorubicin (Sigma, cat. # 44583) were excited with a 488 nm laser to emit fluorescence spectra that were captured within the wavelengths transmitted by 575 ± 26 nm and 530 ± 30 nm filters of a Becton-Dickinson (Franklin Lakes, NJ, USA) LSR II flow cytometer, respectively. Cellular uptake of ^{210}Po -citrate was tracked using a europium tetracycline conjugate (Sigma, cat. #s 203254 for europium and T7660 for tetracycline). Europium tetracycline forms a ternary complex with citrate (EuTc-citrate), which was also excited with a 488 nm laser, and its emission was captured within the wavelengths transmitted by a 610 ± 20 nm filter. Experimental evidence for the appropriateness of using EuTc-citrate as a surrogate for ^{210}Po -citrate was provided in an earlier communication (Figure 3A in Akudugu et al. 2011). Flow cytometry was used to quantify their mean fluorescence intensity (MFI) per cell, $\langle I \rangle$, as a function of the concentration of the agent in the cell culture medium. The net mean fluorescence intensities per cell, $\langle I \rangle_{\text{net}}$, were determined by subtracting mean *control* autofluorescence $\langle I \rangle_{\text{control}}$ (Akudugu et al. 2011):

$$\langle I \rangle_{\text{net}} = \langle I \rangle - \langle I \rangle_{\text{control}} \quad (1)$$

The surviving fraction SF of cells exposed to the agent was assessed with a clonogenic survival assay and plotted as a function of appropriate independent variables including extracellular concentration, $\langle I \rangle_{\text{net}}$, absorbed dose (Gy), and mean cellular activity (mBq/cell) (Akudugu et al. 2011). The resulting survival curves were of a 1- or 2-component exponential form. Analogous to the cellular activity and absorbed dose required to achieve 37% survival, a_{37} and D_{37} , the net mean lethal fluorescence intensity of the drug required to achieve 37% survival, $\langle I \rangle_{\text{net},37}$, was defined similarly and obtained from plots of SF versus $\langle I \rangle_{\text{net}}$ (Akudugu et al. 2011).

Predicting cell survival based on Monte Carlo analysis of cellular fluorescence intensity

Two approaches to modeling the surviving fraction of cells were undertaken in the present study. The first approach, depicted in Figure 1, uses a Monte Carlo simulation that uses experimental individual cell fluorescence intensities I_i for the agent under consideration.

The flow cytometry data for citrate, daunomycin, and doxorubicin, consisting of fluorescence intensities emitted by individual cells in a population after incorporation of a fluorescent agent ($I_1, I_2, I_3, \dots, I_i$), were exported into Microsoft Excel (Redmond, WA, USA) spreadsheets. These raw values were normalized to $\langle I \rangle_{net}$ as per Equation (2).

$$I'_i = I_i \left(\frac{\langle I \rangle_{net}}{\langle I \rangle} \right), \quad (2)$$

where $\langle I \rangle = \frac{1}{N} \sum_{i=1}^N I_i$, and N is the number of cells analyzed. The cytotoxicity of a therapeutic agent in a given cell is assumed to be exponentially related to the cellular uptake of the agent. Exponential functions are widely used to model the probability of cell death following cytotoxic insults from ionizing radiation (Howell and Neti 2005) and chemicals (Boucher and Yakovlev 1997). Accordingly, the survival probability P_i of the i^{th} cell with normalized fluorescence intensity, I'_i , may be expressed as:

$$P_i(I'_i) = e^{\frac{-I'_i}{\langle I \rangle_{net,37}}}, \quad (3)$$

The resulting probability for each cell was compared with a random number generated from a uniform probability distribution by Excel, $0 < RAND_i < 1$, and a binary value was assigned to the survival s_i of the i^{th} cell:

$$s_i = \begin{cases} 0 & \text{dead cell} & RAND_i > P_i(I'_i) \\ 1 & \text{live cell} & RAND_i \leq P_i(I'_i) \end{cases} \quad (4)$$

A new random number was generated for each cell. This type of random number approach to determining the fate of each cell was also used in our recent communication (Rajon et al. 2011). Therefore, the surviving fraction of a population of N cells *treated* with a given concentration of an agent that yields net mean fluorescence intensity per cell, $\langle I \rangle_{net}$, may be expressed as:

$$SF(\langle I \rangle_{net}) = \frac{1}{N} \left(\sum_{i=1}^N s_i \right) \quad (5)$$

Care must be exercised when $\sum_{i=1}^N s_i$ is small because the statistical uncertainty of the Monte Carlo calculation of SF is high under such circumstances. This occurs at high agent concentrations that cause low surviving fractions. This is best circumvented by analyzing a

larger number of cells. A less preferable alternative is to run additional simulations with new random number sequences and average the results.

Predicting cell survival based on mean cellular uptake

The second approach uses the same Monte Carlo approach for determining the fate of each cell; however, it is assumed that every cell in the population contains the same amount of drug. That is, each cell is assigned a fixed net mean fluorescence $\langle I \rangle_{net}$ which, in essence, corresponds to the case wherein the lognormal shape parameter $\sigma \rightarrow 0$. In this instance, the probability of survival of the i^{th} cell is given by:

$$P_i(\langle I \rangle_{net}) = e^{-\frac{\langle I \rangle_{net}}{\langle I \rangle_{net,37}}} \quad (6)$$

The surviving fraction of the population is obtained using the same Monte Carlo method described above except that Equation (6) was used to calculate the probability of survival.

Results

Predicting cell survival based on Monte Carlo analysis of cellular fluorescence intensity

To assess the capacity of Monte Carlo simulation of cell death and survival from cellular fluorescence data acquired by flow cytometry, the procedure depicted in Figure 1 was employed. Only the net mean *lethal* fluorescence intensity, $\langle I \rangle_{net,37}$ and the measured fluorescence intensity in each cell of the treated population were needed to theoretically model the surviving fraction following treatment by each drug. These were both experimentally determined in our earlier communication (Akudugu et al. 2011). Figure 1A-C shows the log-normal nature of the distribution of measured fluorescence intensities following treatment with graded concentrations each drug. Figure 2A is a dot plot that represents the fluorescence intensities I_i for cells treated with 0 or 5 μM daunomycin. Application of Equation (3) to the two cell populations in Figure 2A yields Figure 2B which

represents the survival probabilities $P_i(I'_i)$ of each cell in populations treated with 0 or 5 μM daunomycin. Here, survival probability is directly proportional to bubble size, whereby a cell associated with a large bubble is expected to have a greater chance of surviving than one corresponding to a small bubble. By generating a random number $RAND_i$ between 0 and 1 (Figure 1, STEP 3), and comparing it with the survival probability $P_i(I'_i)$, the fate (s_i) of each cell was determined according to Equation (4) (Figure 1, STEP 4). Figure 2C is a plot of live and dead cells for treated and untreated cell populations. The surviving fraction of cells, $SF(\langle I \rangle_{net})$, based on normalized individual fluorescence intensities I'_i , were then calculated using Equation (5) (Figure 1, STEP 5). To demonstrate the transition from nearly all live cells to nearly all dead cells as the agent concentration is increased, similar dot plots of individual cell survivals s_i are presented for three different concentrations of each agent (Figure 3).

The process described above for determining the surviving fraction $SF(\langle I \rangle_{net})$ was carried out for each of the cell populations which were treated with 0.1-3 mM citrate. The resulting theoretically modeled surviving fractions $SF(\langle I \rangle_{net})$ are plotted in Figure 4A for ^{210}P -

citrate, along with the experimental clonogenic survival data obtained previously (Akudugu et al. 2011). This process was repeated for daunomycin and doxorubicin to create the theoretical data points in Figures 4B and 4C, respectively. The Monte Carlo simulated cell survival is in good agreement with colony forming ability of V79 cells after treatment with the cytotoxic agents.

Predicting cell survival based on mean cellular uptake

Survival curves based on Monte Carlo analysis wherein each cell in the population was assumed to contain the same amount of drug are presented as straight, dashed lines in Figure 4. These provide an important point of comparison with the Monte Carlo model that accounts for the lognormal distribution of cellular uptake of the agent.

Discussion

Nonuniform distribution of radioactivity and its impact on absorbed dose distribution and biological response has been a topic of considerable interest to nuclear medicine for decades (Rossi and Ellis 1952, Wessels and Rogus 1984, Humm 1986, Howell et al. 1989, Makrigiorgos et al. 1990, Goddu et al. 1994, O'Donoghue 1999, Pinto and Howell 2007). Similarly, the implications of nonuniform distributions of chemotherapeutics, and other non-radioactive drugs, have also been discussed within the context of drug design (Durand 1981, Luk and Tannock 1989, Aroui et al. 2010). However, the lognormal nature of cellular incorporation of chemotherapy drugs, radiopharmaceuticals, and radiochemicals has been recognized only recently (Kvinnslund et al. 2001, Neti and Howell 2006, 2008, Akudugu et al. 2011, Rajon et al. 2011). This heterogeneity in agent distribution among cell populations makes estimation of absorbed doses and intracellular drug concentrations, on a cell-by-cell basis, difficult. As a consequence, predictions of biological response have generally been restricted to the use of mean cellular activity or mean cellular drug uptake and therefore generally fail to predict cell survival over several logs of kill (Neti and Howell 2004, Howell and Neti 2005). For a number of radiochemicals, dose-response curves have been shown to saturate with a less than proportionate reduction in cell survival as the mean cellular activity increases (Humm and Chin 1993, Charlton et al. 1994, Neti and Howell 2003, 2004, 2006, 2008, Howell and Neti 2005). In a recent study, a similar tailing in cell survival was observed for the chemotherapeutic drugs, daunomycin and doxorubicin (Akudugu et al. 2011). This saturation phenomenon has been attributed to the lognormal distribution of agent uptake among cell populations. Within this context, the cells that are at the lower end of the agent distribution profile will have higher than expected chance of surviving, than the chance based on the mean cellular agent concentration.

As demonstrated earlier (Kvinnslund et al. 2001), the overall biological response must be influenced by the magnitude of the mean cellular drug uptake and the degree of heterogeneity in agent distribution. Therefore, a change in the capacity of an agent to sterilize a cell population is related to both the change in width of the distribution and the peak-shift as the agent concentration increases (Figure 1A-C). While the former is a measure of the broadness of a distribution profile of the agent among a cell population and can be represented by the lognormal shape parameter, σ , the latter is a shift in the lognormal scale

parameter μ which is an indication of cells accumulating increasing levels of the agent (Neti and Howell 2006). Changes in σ are prognostic of whether a survival curve will exhibit saturation and may guide in the selection of agents for multimodality cocktail design by providing information on agent concentrations at which the first component of cell kill ends (Akudugu et al. 2011). However, the shape parameter only describes the agent distribution profile of the cell population as a whole, but does not provide information on the fate of individual cells of the population.

The present study used a Monte Carlo approach to simulate the fate of each cell based on its experimentally determined drug uptake and used this information to calculate a surviving fraction for the entire cell population. The resulting surviving fractions were compared to experimentally determined values. Two different methods of predicting cell survival following a toxic insult were considered. The first approach addressed the role of individual agent uptake (i.e. cell fluorescence) in cell survival. Figure 2 illustrates how the fate of individual cells can be determined based on their incorporation of a given agent, in this case daunomycin. It is worth noting that the magnitude of a cell's survival probability, per se, is not conclusive as to whether a cell survives or dies. Hence, there is a need to simulate the fate of each cell within the population using Monte Carlo techniques. This is evidenced by the data that are presented in Figure 2B and 2C. Notice that some cells that have higher survival probabilities emerge as dead and vice versa. These and the data in Figure 3 suggest that flow cytometry-assisted Monte Carlo simulation may be a suitable predictor of cytotoxicity. The test of this prediction is found in Figure 4.

In Figure 4, experimentally determined cell survival data are compared with theoretical cell survival data that were simulated by Monte Carlo analysis. However, before applying the Monte Carlo approach to account for the experimental lognormal uptake distributions, it is instructive to first see how this model behaves when each cell is assumed to contain the same uptake (i.e., the mean uptake as per our second method). As expected, when theoretical survival is simulated assuming that each cell in the population contains an agent quantity corresponding to the net mean cellular fluorescence intensity, the resulting survival curve is monoexponential (Figure 4, dashed lines). In this approach, the fact that each cell in the population is assumed to incorporate the same amount of agent implies $\sigma \rightarrow 0$. The data clearly show that, regardless of the agent, the theoretical survival derived from mean cellular fluorescence can recapitulate clonogenic survival only within the first exponential component of cell kill. This is consistent with an earlier caution stating that when the distribution of radioactivity at the multicellular level is nonuniform, the mean absorbed dose to the cell population may not be a suitable predictor of biological response (Neti and Howell 2003).

In contrast to the poor match between experimental data and the mean approach that was discussed above and elsewhere (Neti and Howell 2007), Monte Carlo simulation of cell survival in a manner that accounts for the lognormal uptake distribution provides a very good prediction of clonogenic survival following treatment with ^{210}Po -citrate, daunomycin, and doxorubicin (Figure 4). Moreover, this model accurately predicts the transition between the first and second components of cell killing. This transition coincides with that observed when the lognormal shape parameter is plotted as a function of drug concentration

(Akudugu et al. 2011). It should be noted that in the case of daunomycin, the Monte Carlo approach accurately predicts cell survival at low drug concentrations within the first log of cell kill but there is some deviation from experimental values at high drug concentrations (Figure 4B). Nevertheless, the fit is remarkably good compared with the more conventional approach based on mean drug uptake (Figure 4, dashed lines). As pointed out in earlier publications, approaches based on mean uptake adequately predict the response in the low-dose realm, but fail to predict the upward trend in the survival curve that arises as a consequence of the lognormal distribution of drug uptake (Howell and Neti 2005).

Of particular importance in this study is that the flow cytometry-assisted Monte Carlo simulation of cell survival requires knowledge of only the initial slope of the dose-response curve (i.e., $<I>_{net,37}$) and the uptake distribution of the radiopharmaceutical or drug. With these two pieces of information, the entire clonogenic survival curve can be recapitulated including both the 1- or 2-component exponential shapes. The approach should apply equally well for drugs that are not likely to be characterized by a lognormal uptake distribution such as Hoechst 33342, whose uptake is directly proportional to DNA content.

When the methods presented herein are applied more broadly, special attention will have to be given to the process of normalizing the fluorescence data obtained by flow cytometry. The measured fluorescence intensities are dependent on flow cytometry hardware (e.g., laser wavelength and intensity), settings (e.g., amplification), etc. Given that the fluorescence intensity is ultimately related to drug uptake in terms of quantities such as mass (g) and/or activity (Bq), there will be a need to implement calibrations for these quantities. The mean activity per cell can be readily measured with high accuracy and precision using standard radiation detection devices. This information, along with the distribution of cellular fluorescence intensities, provides detailed knowledge of the activity in each cell of the population. Furthermore, calibration with drugs with known specific activity (Bq/g) can provide detailed knowledge of the mass of drug in each cell of the population. Accordingly, as discussed above, survival probabilities might be represented by $P_i = e^{-\frac{m_i}{<m>_{37}}}$ and $e^{-\frac{a_i}{<a>_{37}}}$, respectively, where the mass of drug in the cell, $m_i = \xi I'_i$ and/or the amount of radioactivity in the cell $a_i = \kappa I'_i$. The constants ξ and κ represent the slopes of plots of $<m>$ and $<a>$ versus $<I>_{net}$, respectively. The latter was provided for ^{210}Po -citrate elsewhere (Akudugu et al. 2011). These probabilities would be independent of flow cytometry hardware and instrument settings.

Also not addressed by this study are the underlying reasons why the experimental and Monte Carlo derived clonogenic survival curves deviate most from those for an average concentration of agent at the higher concentrations. In the realm of chemotherapy, this is often ascribed to resistant subpopulations that may express high levels of the multidrug resistant protein MDR1 (Kovtun et al. 2010). One function of this protein is to facilitate the active removal of toxins from the cell, thereby foiling its therapeutic intent. Low cellular uptake by some cells within a population is also especially important for receptor-targeted agents such as radiolabeled antibodies used for radioimmunotherapy (Kvinnslund et al. 2001). The number of receptors on a given cell can vary widely over a cell population such that sublethal activity may be taken up by a subpopulation. The flow cytometry assisted

Monte Carlo method presented in the present article can be extremely useful in modeling the consequence of such nonuniformities, thereby reducing the level of experimental effort that is needed to optimize a therapy. Furthermore, a variety of other capabilities can be built into the model to account for other toxic insults to the cell population such as cross-dose received from radiations emitted by neighboring cells (Rajon et al. 2011), and radiation- or chemically-induced bystander effects (Howell and Bishayee 2002, Xue et al. 2002).

Conclusion

This study evaluates the use of flow cytometry-assisted Monte Carlo simulation to predict clonogenic survival. It is demonstrated that Monte Carlo simulation using cellular agent incorporation based on individual cell fluorescence intensity of therapeutic agents is a suitable predictor of cell survival. This flow cytometry-based approach, which takes explicit account of the lognormal distribution of cellular uptake of the agents, offers a rapid means for determining treatment response on a cell-by-cell basis, and may be invaluable in the selection of agents for the design of highly effective therapeutic cocktails that are capable of targeting the diversity in tumor cell populations (Gerlinger and Swanton 2010, Akudugu et al. 2011). Such cocktails can be created not only for treatment of cancer, but also for infectious diseases (Saylor et al. 2009) and other diseases that may be amenable to targeted therapies. Furthermore, this single-cell Monte Carlo technique can be used to resolve difficulties encountered when attempting to predict biological response at the multicellular level using macroscopic mean agent doses (Neti and Howell 2003, 2004, Howell and Neti 2005).

Acknowledgments

This work was supported in part by NIH Grant Number R01 CA083838-09. The content is solely the responsibility of the author and does not necessarily represent the official views of the National Cancer Institute or the National Institutes of Health. We thank Sukwinder Singh and Dana Stein for their assistance in the New Jersey Medical School Flow Cytometry Core Facility, and Prasad Neti for his insightful comments.

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Figure 1.

Flow chart of Monte Carlo procedure for determining fraction of surviving cells based on cellular fluorescence intensity profiles of the incorporated agent. In STEP 1, flow cytometry was used to obtain fluorescence intensity of EuTc-citrate (^{210}Po -citrate), daunomycin, and doxorubicin in individual V79 cells in a suspension culture. The distribution of measured cellular fluorescence intensities, adapted from (Akudugu et al. 2011), is shown in (A) 0.1–3 mM citrate, (B) 0–10 μM daunomycin, or (C) 0–10 μM doxorubicin. After calculating the probability of survival $P_i(I'_i)$ for the i^{th} cell based on the normalized fluorescence intensity I'_i (STEP 2), a random number RAND_i ($0 < \text{RAND}_i \leq 1$) was generated as depicted in STEP 3 by the dice. If $\text{RAND}_i < P_i(I'_i)$, then the cell was scored as a survivor, otherwise it was considered dead (STEP 4). A surviving cell is represented by a blossoming tree with leaves, while a dead cell is depicted by a tree without leaves. By repeating STEPS 1–4 for every cell in each population, the surviving fraction for any sample population was calculated as illustrated in STEP 5.

Figure 2.

Graphical depiction of the steps used in Monte Carlo simulation of the surviving fraction of cells. For brevity, data are shown for only the first 2000 cells that were analyzed by the flow cytometer. Cells were treated with 0 μM (green) or 5 μM (red) daunomycin. (A) Dot-plot of cellular fluorescence intensity I_i versus i (STEP 1, Figure 1). (B) Plot of probability $P_i(I'_i)$ that the i^{th} cell survives versus i (STEP 2, Figure 1). As depicted in the shaded region, P_i is directly proportional to the size of the corresponding bubble (Microsoft Excel software, Redmond, WA, USA). Note that cells with a negligible probability of survival are not visible due to their minute bubble sizes. (C) Plot of cell fate versus i (STEP 4, Figure 1). Large bubbles represent survivors and small bubbles represent dead cells. Data illustrating the effect of agent concentration on cell fate is presented in Figure 4.

Figure 3.

Monte Carlo simulation of fate of V79 cells based on agent uptake in individual cells. Large bubbles represent survivors and small bubbles represent dead cells. (A) To evaluate the relation between the extent of ^{210}Po incorporation and cell survival, cells were treated with 0.1 mM (green), 2 mM (yellow), and 3 mM (red) citrate for 30 min prior to flow cytometric analysis. (B) Cells were treated with 0 μM (green), 2.5 μM (yellow), and 5 μM (red) daunomycin for 2.5 h before flow cytometry. C. Cells were exposed to 0 μM (green), 5 μM

(yellow), and 8 μ M (red) doxorubicin for 2.5 h prior to flow cytometric analysis. All agent exposures were performed in suspension cell cultures.

Figure 4.

Comparison of Monte Carlo simulated cell survival (colored symbols) with experimental clonogenic cell survival (black symbols) of V79 cells after treatment with (A) ^{210}Po -citrate, (B) daunomycin, or (C) doxorubicin. The experimental data were reported previously; the surviving fraction for ^{210}Po -citrate are plotted against mean absorbed dose to the cell nucleus, mean intracellular ^{210}Po activity, and mean fluorescence intensity of the europium tetracycline-citrate complex (Akudugu et al. 2011). Dashed lines represent Monte Carlo simulations of cell survival when every cell in the population is assumed to contain the same amount of drug that corresponds to the mean drug uptake for the respective extracellular concentration (i.e., net mean fluorescence intensity). Error bars represent the SE for $\langle I \rangle_{\text{net}}$ based on fluorescence data from two and three independent experiments for daunomycin and doxorubicin, respectively. Error bars for ^{210}Po citrate data are smaller than the symbols.