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A Method to Predict Response of Cell Populations to Cocktails of Chemotherapeutics and Radiopharmaceuticals: Validation with Daunomycin, Doxorubicin, and the Alpha Particle Emitter ^{210}Po

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Abstract

There is considerable interest in the use of α -emitting radionuclides in radioimmunotherapy. However, the high toxicity of α -emitting radionuclides often does not permit administration of high activities for fear of normal tissue toxicity. Accordingly, targeting procedures need to be optimized for improved tumor control and minimized normal tissue toxicity. To guide design of effective cocktails of α -emitting radiopharmaceuticals and chemotherapy drugs, approaches that can predict biological response of a cell population on a cell-by-cell basis are needed.

Methods—Cells were concomitantly treated with the α -particle emitting radiochemical ^{210}Po -citrate and daunomycin, or with ^{210}Po -citrate and doxorubicin. The responses of the treated cell populations were measured with a colony forming assay. The nonuniform cellular incorporation of the radiochemical and drugs was determined *simultaneously* on a cell-by-cell basis using flow cytometry. Monte Carlo methods were used to simulate cell survival on the basis individual cell incorporation of each cytotoxic agent and validated by direct comparison with the experimental clonogenic cell survival.

Results—Both daunomycin and doxorubicin enhanced the toxicity of the α -particles with a magnitude greater than expected based on single-agent toxicities. Cell survival obtained by Monte Carlo simulation was in good agreement with clonogenic cell survival for the combination treatments.

Conclusion—Flow cytometry assisted Monte Carlo simulations can be used to predict toxicity of *cocktails* of α -emitting radiopharmaceuticals and chemotherapy drugs in a manner that takes into account the effects of nonuniform distributions of agents within cell populations.

Keywords

radionuclide therapy; chemotherapy; Monte Carlo; alpha particle; drug cocktail

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INTRODUCTION

Over the past three decades, a significant effort has been directed toward achieving improvements in treatment of cancer with radioimmunotherapy [1–3], radiolabeled chemotherapy agents [4–8], and a combination of chemotherapy with radioimmunotherapy [9, 10]. Although considerable successes have been realized with targeted radionuclide therapy and translated into front-line therapies [2], the full potential of this therapeutic modality has yet to be realized. There has been more success in radioimmunotherapy of hematologic malignancies [11–14] than in solid tumors [9, 15–19]. It has been suggested that 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 nonuniform [20–23] and can be lognormal [23–25]. Interestingly, antibodies (and their fluorochrome or radionuclide labeled counterparts) often target a given cell population in a lognormal manner as evidenced by the relatively symmetric fluorescence intensity histograms that are commonly observed in flow cytometry studies with these proteins [23]. In such distributions, some malignant cells may take up very little or no therapeutic agent. This can lead to minimal or no toxic effects on such populations depending on the extent of cross-irradiation from nearby cells that have taken up radiopharmaceuticals [21, 26, 27]. As such, repopulation of under-targeted subpopulations of cells in either the primary tumor or in micrometastases may lead to therapeutic failure [21].

Interest in the use of α -emitting radionuclides in radioimmunotherapy has grown significantly [28–33]. A critical review on the dosimetry aspects of targeted α -emitter therapy was recently published by Sgouros et al. [29]. However, a major unresolved concern is that the extremely high toxicity of α -emitting radionuclides does not generally permit high administered activities. Accordingly, targeting procedures for this modality need to be optimized to minimize normal tissue toxicity. One potential approach to achieving this is a combination of radioimmunotherapy and chemotherapy [34]. Such approaches seek not only to target all malignant cells, but also significantly reduce the amount of each constituent of the cocktail. Recent studies have shown that a combination of radioimmunotherapy (^{213}Bi -anti-CD45) and chemotherapy can kill chemo- and radioresistant leukemia cells [35]. Although such cocktail approaches appear promising, a major limitation is the difficulty in predicting the efficacy of given cocktail for a given patient. This problem may be related, in part, to lack of knowledge regarding the distribution of the agents amongst the targeted cell population. Therefore, developing methods to determine the distribution of a cocktail of cytotoxic agents among a targeted cell population at the single-cell level and to predict their effect on the population, may advance the cocktail approach to a stage whereby all malignant cells can be reliably targeted.

Past studies have shown that ^{210}Po -citrate [24, 25, 36], daunomycin [24], and doxorubicin [24] are lognormally distributed and that this type of nonuniform distribution leads to incomplete sterilization of the treated population [24, 37]. The present study builds on these past studies with single agents [24, 37], by investigating the cytotoxicity of combinations of these agents. It also describes how the toxicity of the combination treatments can be predicted by extending and validating our Monte Carlo simulation approach, previously applied only to single agents [37], to the more complex scenario involving multiple agents. This method may be useful for selecting agents for therapeutic cocktails that are capable of targeting the diversity in tumor cell populations [24, 38], infected cells [39, 40], and other diseases that may be amenable to targeted therapies.

MATERIALS AND METHODS

Cell Culture and Clonogenic Survival Measurements

All media and supplements used in this study were from Gibco® (Carlsbad, CA). Two different formulations of minimum essential media (MEMA and MEMB) were used to culture Chinese hamster V79 lung fibroblasts as previously described [41]. MEMA contained minimum essential medium (Gibco cat #11095) supplemented with 10% heat-inactivated fetal calf serum (Gibco cat # 10437, lot # 539574) with 2 mM L-glutamine, 50 units/ml penicillin, and 50 µg/ml streptomycin. For routine maintenance, cells were grown as monolayers in Falcon 25-cm² tissue culture flasks (Becton Dickinson (BD), cat # 353082) at 37°C with 5% CO₂ and 95% air, and were subcultured twice weekly. For experiments, V79 cells (passages 4–11) were transferred into Falcon 225-cm² flasks (BD, cat # 353138) and were used on reaching 80%–90% confluence. The cells were trypsinized and were resuspended at 2×10⁶ cells per mL in MEMB (suspension culture medium) which was calcium-free MEM (Gibco cat #11380) with the same supplements as MEMA. Aliquots of 1 mL were transferred into sterile Falcon™ 17×100 mm polypropylene round-bottom culture tubes (BD, cat # 352018) and incubated on a rocker-roller (Thermo Fisher) for 3 h at 37°C with 5% CO₂ and 95% air.

After this conditioning period, cells were treated with ²¹⁰Po-citrate (0–250 kBq/mL) for 30 min while being incubated on the rocker-roller at 37°C with 5% CO₂ and 95% air, washed twice, treated with daunomycin (Sigma, cat # D8809) or doxorubicin (Sigma, cat # 44583), and the cell cultures were returned to the rocker-roller for 2.5 h. ²¹⁰Po has a physical half-life of 138 d and emits a single 5.3 MeV α-particle [42]. The ²¹⁰Po-citrate was prepared as previously described [25]. ²¹⁰PoCl₄ in 2 M HCl was obtained at 370 MBq/mL from Eckert and Ziegler Isotope Products (cat # 6310). The ²¹⁰PoCl₄ solution was mixed with 1 M sodium citrate in the ratio of 1:7 (final pH, 5.8) and was diluted with MEMB to a volume of 4 mL (final pH, 6.9). The final concentrations of daunomycin and doxorubicin corresponded to that required to achieve 10% cell survival and were predetermined to be 0.63 and 2.50 µM, respectively [24].

After drug treatment, cells were washed twice with MEMB, resuspended in 5 mL of MEMB, and passed 5 times through a 21-gauge needle. Cell concentrations were measured with a Beckman Coulter Model Z2 (Brea, CA). Aliquots (500 µL) of the cell suspension were transferred to vials, mixed with 5 mL Ecolume (MP Biomedical, cat # 882470), counted in a Beckman Coulter LS6500 liquid scintillation counter, and the mean activity per cell was determined (counting efficiency, 50%). Aliquots of about 0.5 mL of cells were counted in triplicate for ²¹⁰Po activity. The triplicate measurements kept statistical variations to a minimum. In each experiment, a parallel set of samples treated with ²¹⁰Po-citrate alone were used to determine the toxicity of ²¹⁰Po-citrate in the absence of the drugs. Each sample was then serially diluted with MEMA and plated in Falcon 60×15 mm tissue culture dishes for colony formation. Cultures were incubated for 7 days, and the colonies were fixed in 95% ethanol, stained with 0.01% amido black, washed in tap-water, air-dried, counted, and the surviving fraction of cells relative to controls was determined.

Cellular Incorporation of ²¹⁰Po-Citrate, Daunomycin, and Doxorubicin

The uptake of ²¹⁰Po-citrate and the fluorescent chemotherapy drugs daunomycin and doxorubicin were determined on a cell-by-cell basis by flow cytometric techniques. Briefly, V79 cells (2 × 10⁶ cells/mL) preconditioned as described above were treated with 0–3 mM ²¹⁰Po-free citrate for 0.5 h, washed twice with MEMB, and treated with 0.63 and 2.50 µM of daunomycin and doxorubicin for 2.5 h, respectively. Cells were then washed twice with 10 mM 3-morpholino-propanesulfonic acid (MOPS) buffer (Sigma, cat # M3183), and

processed for flow cytometry as described previously [24]. To detect citrate, cells were resuspended in 1 mL of MOPS buffer containing 100 μM of europium tetracycline (Sigma, cat #s 203254 for europium and T7660 for tetracycline), transferred into 7-mL polystyrene flow cytometry tubes (BD, cat # 352054), and incubated at room temperature ($\sim 22^\circ\text{C}$) in the dark for 30 min. This process creates a europium tetracycline–citrate complex that is detectable by flow cytometry. After washing the cells twice with MOPS buffer, the samples were resuspended in 1 mL of MOPS buffer, passed 5 times through a 21-gauge needle, and subjected to flow cytometry. Measurement of cellular uptake of citrate by flow cytometry was previously shown to correlate directly with cellular uptake of ^{210}Po -citrate and therefore it serves as a suitable surrogate for its cellular uptake [24].

Acquisition and Analysis of the Flow Cytometry Data

Samples—Flow cytometry control samples consisted of cells treated with the following agents: 1) untreated, 2) 3 mM citrate, 3) 0.63 μM daunomycin, and 4) 2.50 μM doxorubicin. Daunomycin + citrate test samples were 0.63 μM daunomycin + 0.1, 0.2, 0.5, 1.0, 1.5, 2.0, 2.5, or 3.0 mM citrate. Doxorubicin + citrate test samples were 2.50 μM doxorubicin + 0.1, 0.2, 0.5, 1.0, 1.5, 2.0, 2.5, or 3.0 mM citrate.

Acquisition—Fluorescence intensity histograms were acquired for each sample using an LSR II flow cytometer (BD). The europium tetracycline–citrate complex, daunomycin, and doxorubicin were excited with a 488 nm laser, and their emission spectra were captured within the wavelengths transmitted by the 610/20, 575/26 and 530/30 filters, respectively. The flow cytometry data files for each acquisition are included as supplemental data (.FCS files). Representative fluorescence intensity histograms for each agent in V79 cells are provided in Ref. [24].

Analysis—FlowJo software (TreeStar) was used to analyze each sample. Dot plots of forward scatter versus side scatter were created to gate cells from debris. Fluorescence intensities were compensated for overlapping emission spectra (Uncompensated, Figure 1; Compensated, Figure 2).

Prediction of Multiple Agent Toxicity

The approach for modeling cell survival using a Monte Carlo simulation based on individual cell fluorescence intensities I_i for a single agent has been described in detail elsewhere [37]. In the present work, when cells were concomitantly treated with two agents, the fluorescence intensities of each agent within each cell of the treated population were measured simultaneously using flow cytometry. These data were used to perform a Monte Carlo analysis to simulate the surviving fraction of cells after treatment with ^{210}Po -citrate + daunomycin and ^{210}Po -citrate + doxorubicin. This process is depicted in Figure 3 and described below for the general case of multiple agents.

The compensated fluorescence intensity data were exported into Microsoft Excel (Redmond, WA) spreadsheets. The compensated fluorescence intensities for the j^{th} agent in the i^{th} cell, I_{ij} , were normalized to $\langle I_j \rangle_{\text{net}}$ for each agent as per Equation (1). This approach was previously shown to work very well for single drug analysis [37].

$$I'_{ij} = I_{ij} \left(\frac{\langle I_j \rangle_{\text{net}}}{\langle I_j \rangle} \right), \quad (1)$$

Where the arithmetic mean fluorescence intensity $\langle I_j \rangle = \frac{1}{N} \sum_{i=1}^N I_{ij}$, and, in the case of ^{210}Po -citrate, correlates with the arithmetic mean initial activity per cell $\langle a_0 \rangle = \frac{1}{N} \sum_{i=1}^N a_i$ [24]. The quantity $\langle I_j \rangle_{net}$ denotes the net mean fluorescence intensity per cell following exposure to the j^{th} agent and is determined by subtracting the arithmetic mean *control* autofluorescence, $\langle I_j \rangle_{control}$, from the arithmetic mean fluorescence intensity per cell in a treated population as defined by Equation (2) [24, 37]:

$$\langle I_j \rangle_{net} = \langle I_j \rangle - \langle I_j \rangle_{control} \quad (2)$$

For ^{210}Po -citrate, a citrate concentration of 0.1 mM, which was found to correspond to a nonlethal cellular activity of 0.03 mBq/cell, was used for *control* autofluorescence as in our previous work [24]. The net mean cellular fluorescence intensity of Agent j that yields 37% survival is denoted $\langle I_j \rangle_{net,37}$ [24]. To account for natural variations in $\langle I_j \rangle_{net,37}$ from experiment to experiment, it is necessary to obtain an $\langle I_j' \rangle_{37}$ for each experiment from a calibration of an experimentally determined surviving fraction. Assuming that the toxicity of the j^{th} agent in a given cell population is exponentially related to the cellular uptake of the agent [24, 37], the surviving fraction of such a population based on its net mean fluorescence intensity, $\langle I_j' \rangle$, is:

$$SF_j = e^{-\langle I_j' \rangle / \langle I_j' \rangle_{37}} \quad (3)$$

For instance, using the net mean fluorescence intensity of a cell population corresponding to

10% cell survival, $\langle I_j' \rangle_{37} = -\frac{\langle I_j' \rangle}{\ln(0.1)}$. At the single-cell level, the survival probability of the i^{th} cell with normalized fluorescence intensity, I_{ij}' , may be expressed as (Figure 3, STEP 2):

$$P_i(I_{ij}') = e^{-I_{ij}' / \langle I_j' \rangle_{37}} \quad (4)$$

Therefore, the survival probabilities for the i^{th} cell when treated with Agent 1 or Agent 2 are given by $P_i(I_{i1}') = e^{-I_{i1}' / \langle I_1' \rangle_{37}}$ and $P_i(I_{i2}') = e^{-I_{i2}' / \langle I_2' \rangle_{37}}$, respectively.

To model cell survival following treatment with a cocktail of two agents, we hypothesize that a cell may die due to Agents 1 and 2 working independently or interactively. The survival probability $P_i(I_{i1}', I_{i2}')$ of the i^{th} cell may be represented by:

$$P_i(I_{i1}', I_{i2}') = (P_i(I_{i1}') P_i(I_{i2}'))^{DMF} = e^{-DMF(I_{i1}' / \langle I_1' \rangle_{37} + I_{i2}' / \langle I_2' \rangle_{37})} \quad (5)$$

where DMF represents a dose modification factor that accounts for interaction of the agents (e.g. protection or synergy). DMF = 1 when there is no interaction between the agents. In a two drug scenario, DMF $\rightarrow$ 1 when the concentration of either drug goes to zero.

The probability calculated with Equation (5) was then compared with a random number, $0 < RAND_i < 1$ (Figure 3, STEP 3), and a binary value was assigned to the survival s_i of the i^{th} cell:

$$S_i = \begin{cases} 0 & \text{dead cell} & RAND_i > P_i(I'_{i1}, I'_{i2}) \\ 1 & \text{live cell} & RAND_i \leq P_i(I'_{i1}, I'_{i2}) \end{cases} \quad (6)$$

A new random number was generated for each cell. This approach to determining the fate of each cell (Figure 3, STEP 4) was also used in our recent communications [37, 43].

Therefore, the theoretical surviving fraction of a population of N cells treated with Agent 1 + Agent 2 is given by (Figure 3, STEP 5):

$$SF(<I_1>_{net}, <I_2>_{net}) = \frac{1}{N} \left(\sum_{i=1}^N S_i \right) \quad (7)$$

Comparison with other Normalization Methods

The arithmetic mean of a lognormal distribution is dependent on the scale and shape parameters of the lognormal distribution [25]. This quantity was used Eqs. (1) and (2) to normalize the fluorescence intensity data. Similar normalization of the fluorescence intensities was also attempted with the net median (i.e. geometric mean) intensity and the mode of the intensity. In addition, the logs of the intensities were also normalized in various ways. However, these approaches did not improve the predictive capacity of the Monte Carlo model beyond what was achieved with the net mean fluorescence intensity approach used in Eqs. (1) and (2).

RESULTS

Toxicity of Cocktail

To evaluate cytotoxicity of combined treatment of V79 cells with ^{210}Po -citrate and daunomycin, or ^{210}Po -citrate and doxorubicin, the surviving fraction of clonogens was plotted as a function of mean cellular uptake of ^{210}Po (Figure 4) and as a function of mean absorbed dose to the cell nucleus, calculated as described previously [24]. When cells were treated with ^{210}Po -citrate alone, at levels sufficient to afford only two logs of kill, the survival curve can be described by a 1-component exponential function. When cells were concomitantly treated with a single drug concentration and varying amounts of ^{210}Po -citrate, the dose-response curves followed a 2-component exponential function. This two-component structure likely emerged because of the strong two-component structure observed when these cells were treated with each drug alone [24]. The data corresponding to the combined treatments were then corrected for drug toxicity, by dividing the surviving fraction for each datum point by the surviving fraction for drug alone ($SF = 0.10$), and replotted in Figure 4. The resulting cell survival curves, corrected accordingly for daunomycin and doxorubicin toxicity, emerged below those obtained for ^{210}Po -citrate alone. This indicates that daunomycin and doxorubicin *enhance* the toxicity of ^{210}Po -citrate in V79 cells. As illustrated in Figure 4A, the enhancement afforded by daunomycin was independent of radiation absorbed dose beyond about 0.5 Gy ($\sim 0.09 \text{ mBq/cell}$). On the other hand, the increased kill afforded by doxorubicin showed a dose-dependent increase (Figure 4B). These enhancements were quantified in the form of a dose modification factor (DMF), in this case a DMF based on areas under survival curves [44]. To obtain the DMF, the cell survival data were replotted on a linear scale (Figure 5). The survival curve for ^{210}Po -citrate alone was compared with the survival curve for ^{210}Po -citrate + daunomycin, corrected for daunomycin toxicity (Figure 5A). The corresponding plots for doxorubicin are shown in Figure 5B. The area A under each curve was determined and the ratio of the two areas was taken to be the dose modification factor: $DMF^{210\text{Po-citrate+agent}} = A(^{210}\text{Po-citrate})/$

$A(^{210}\text{Po-citrate}+\text{agent, corrected for agent toxicity})$. The resulting experimental DMF values were $DMF(^{210}\text{Po-citrate}+\text{daunomycin}) = 2.6$, and $DMF(^{210}\text{Po-citrate}+\text{doxorubicin}) = 1.7$.

Predicting Cell Survival Based on Monte Carlo Analysis of Cellular Fluorescence Intensity

The procedure depicted in Figure 3 was used to assess the capacity of flow-cytometry assisted Monte Carlo simulation to recapitulate cell death and survival after exposure to $^{210}\text{Po-citrate} + \text{daunomycin}$ or $^{210}\text{Po-citrate} + \text{doxorubicin}$. Following STEPS 2–4 of Figure 3, application of Equations 4–6 yields Figure 6 which is a plot of live and dead cells for fixed daunomycin concentration and varying concentrations of $^{210}\text{Po-citrate}$, where large bubbles represent survivors ($s_i = 1$) and dots represent dead cells ($s_i = 0$). The surviving fraction of a given cell population, based on normalized individual fluorescence intensities, I'_{ij} , was then calculated using Equation 7 (Figure 3, STEP 5). The symbols in Figure 7 represent the theoretically modeled surviving fractions for $^{210}\text{Po-citrate} + \text{daunomycin}$ and $^{210}\text{Po-citrate} + \text{doxorubicin}$. The theoretical data are superimposed on curves representing least squares fits to the experimental clonogenic survival data that were shown in Figure 4. The open symbols were obtained assuming independent action of the agents ($DMF=1$), while closed symbols represent modeled cell survival when the agents are considered to interact ($DMF = 2.6$ and 1.7 for daunomycin and doxorubicin, respectively). The Monte Carlo simulation that implemented agent-interaction appears to be in good agreement with the experimental data. In an attempt to quantify the goodness of the agreement, the experimental DMF values used in the Monte Carlo simulation were manually varied to minimize the sum of the squares of the difference in the logs of the corresponding experimental and theoretical values of SF (i.e. $\sum (\ln SF_{\text{theoretical}} - \ln SF_{\text{experimental}})^2$). The sum of the squares was minimized when $DMF = 2.7$ and 2.1 for daunomycin and doxorubicin, respectively.

DISCUSSION

Lognormal distributions of therapeutic agents within cell populations may play an important role in the possible modification of α -particle radiotoxicity by chemotherapy drugs. Figure 4 provides experimental evidence that treatment of Chinese hamster V79 cells with a cocktail of $^{210}\text{Po-citrate}$ and a chemotherapy drug (daunomycin or doxorubicin) causes cytotoxicity greater than expected based on the lethality of the agents when used alone. These data show that when the effect of each chemotherapy drug is corrected for, the corrected survival curves do not coincide with that for the $^{210}\text{Po-citrate}$ alone treatment (Figure 4 dotted lines and Figure 5). The corrected curves emerge below the $^{210}\text{Po-citrate}$ survival curve. This is indicative of either a chemical enhancement of the radiotoxicity of the 5.3 MeV α -particles emitted by ^{210}Po , or that cells that are not adequately labeled with $^{210}\text{Po-citrate}$ (e.g. low end of the lognormal uptake distribution) are killed by the chemotherapy drug. As discussed below, the flow-cytometry assisted Monte Carlo simulation of cell survival can shed light on distinguishing between these two possibilities.

In a preceding study, it was demonstrated that flow cytometry-assisted Monte Carlo simulation of cell survival based on individual cellular fluorescence intensities after exposure to a single drug can recapitulate cytotoxicity of the drug as measured by colony forming ability [37]. This model predicts cell survival in a manner that accounts for the lognormal uptake distribution of cytotoxic agents. In the present study, the model is applied to agent-incorporation data obtained after treating cells with a *cocktail* of $^{210}\text{Po-citrate} + \text{daunomycin}$ or $^{210}\text{Po-citrate} + \text{doxorubicin}$. The test of the capacity of this approach to predict the cytotoxicity of a combination therapy is presented in Figure 7 where experimentally determined cell survival curves (solid curves) are compared with theoretical cell survival as simulated by Monte Carlo analysis (symbols). When it is assumed that the

agents act independently ($DMF=1$), the model fails to predict clonogenic cell survival (Figure 7, open symbols). Had the model been able to predict the survival when $DMF=1$, one could conclude that the differences in the curves in Figure 5 were solely related to the lognormal distributions of the agents. Therefore, it would appear that the enhanced combined toxicity of the two agents is consistent with their interacting in some way. This is supported by the Monte Carlo simulations, wherein the agents are assumed to interact (i.e. $DMF > 1$), which predict cell survival quite well at all intracellular activities of ^{210}Po (Figure 7, closed symbols). This suggests that, at the levels of cell kill observed in the present study, the chemotherapy agents provide a chemical enhancement of the ^{210}Po α -particle radiotoxicity in V79 cells rather than killing cells that are inadequately labeled with ^{210}Po -citrate. The latter possibility may become important at very high levels of cell kill (i.e. 5 or 6 logs of kill).

Although the latter approach accurately predicts the experimental toxicity of the ^{210}Po -citrate + daunomycin/doxorubicin cocktails based only on knowledge of the *initial* slope of the dose-response curves for each agent (i.e. $\langle I_j \rangle_{37,net}$) and the cellular uptake distribution of the ingredients of the cocktail [37]. This can be extremely helpful in designing more effective cocktails for targeted therapy; these cocktails may consist of a sizeable number of agents. However, what remains unresolved is how to describe the DMF when calculating the survival probabilities for multiple agents. As indicated in Eq. 8, we anticipate that the survival probability will take on a form similar to the two-agent case in Eq. 5, noting that the DMF may depend on the intracellular concentrations of the various agents.

$$P_i(I'_{i1}, I'_{i2}, \dots, I'_{iK}) = \left(\prod_{j=1}^K e^{-I'_{ij}/\langle I_j \rangle_{37}} \right)^{DMF(I'_{i1}, I'_{i2}, \dots, I'_{iK})} \quad (8)$$

The agents may interact as in the present study, however, one can readily envision agents that do not interact significantly. Cocktails of agents that do not interact, but have non-overlapping lognormal distributions of cellular uptake such that cells with low amounts of one agent have high amounts of another agent, are expected to be modeled more easily.

The use of α -emitting radionuclides in radioimmunotherapy has gained considerable interest [28–33], but the relatively high potency of α -particles often limits the amount of activity that can be administered. To therapeutically benefit from the potency of α -particles and yet maintain low normal tissue toxicity, the use of low doses of cocktails of α -particle based radioimmunotherapeutics and chemotherapy drugs that adequately target all malignant cells with at least one or more agents is warranted. Radioimmunotherapy has been strongly advocated [10, 34, 45]. Yet, only few studies have investigated this approach with α -particle emitters [29, 35]. Needless to say, predicting response to combined radionuclide therapy and chemotherapy drugs on a cell-by-cell basis may be of great help to dissect mechanisms involved with drug interaction and improve the design of more effective cocktails for targeted therapy. With this in mind, it is possible that agents previously discarded on the basis of single-agent toxicity, may become key ingredients in a cocktail by virtue of their capacity to target a few cells that only incorporate small amounts of the primary drug.

CONCLUSION

This study provides experimental evidence for the enhancement of α -particle toxicity in Chinese hamster V79 cells by two chemotherapy drugs. It further evaluates the capacity to predict clonogenic survival after multi-modality therapy, using flow cytometry-assisted Monte Carlo simulation. It is demonstrated that Monte Carlo simulation using cellular agent incorporation based on individual cell fluorescence intensities of therapeutic agents is a

suitable predictor of cell survival. This model accounts for the lognormal distribution of cellular uptake of the agents, and is capable of predicting treatment response on a cell-by-cell basis. The approach may be valuable in the selection of agents for the design of effective α -particle based therapeutic cocktails that are capable of targeting the diversity in tumor cell populations.

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FIGURE 1.

Uncompensated flow cytometry dot plots for control samples consisting of cells treated with **A)** 0.63 μ M daunomycin, **B)** 0.63 μ M daunomycin + 3 mM citrate, **C)** untreated, and **D)** 3 mM citrate,

FIGURE 2.

Compensated flow cytometry dot plots for control samples consisting of cells treated with **A)** 0.63 μM daunomycin, **B)** 0.63 μM daunomycin + 3 mM citrate, **C)** untreated, and **D)** 3 mM citrate, FlowJo software was used to compensate for overlapping emission spectra.

FIGURE 3.

Flow chart of Monte Carlo procedure for determining fraction of surviving cells based on cellular fluorescence intensity distributions that arise after treatment of cells with a cocktail of two therapy agents (Agent 1 and Agent 2). In STEP 1, flow cytometry is used to obtain a dot-plot of the treated cells. STEP 2 calculates the probability $P = (I'_{i1}, I'_{i2})$ that the i^{th} cell survives treatment with Agents 1 and 2, based on the respective normalized fluorescence intensities I'_{i1} and I'_{i2} . A random number $RAND_i$ ($0 < RAND_i < 1$) is then generated as depicted in STEP 3 by the dice. If $RAND_i < P(I'_{i1}, I'_{i2})$, then the cell is scored as a survivor, otherwise it is considered dead (STEP 4). By repeating STEPS 1–4 for every cell, the surviving fraction for any sample population is calculated as illustrated in STEP 5.

FIGURE 4.

Surviving fraction of V79 cells after treatment with graded amounts of ^{210}Po -citrate in the absence or presence of: (A) 0.63 μM daunomycin or (B) 2.50 μM doxorubicin. Colors represent independent experiments ($\circ$ ^{210}Po -citrate; $\blacksquare$ ^{210}Po -citrate + daunomycin; $\blacktriangle$ ^{210}Po -citrate + doxorubicin). Dashed lines represent least-squares fits of data for ^{210}Po -citrate to a 1-component exponential function. Solid curves represent least-squares fits of data for the combined treatment to 2-component exponential functions. Correction of the combined treatment curves for drug toxicity yielded the dotted curves. Horizontal and vertical error bars represent SE of mean cellular activity and surviving fraction of triplicate measurements, respectively. Some error bars are smaller than the symbols.

FIGURE 5.

Determination of dose modification factor DMF. Select data from Figure 4 were replotted on a linear ordinate axis: **A)** ^{210}Po -citrate alone and ^{210}Po -citrate+daunomycin, **B)** ^{210}Po -citrate alone and ^{210}Po -citrate+doxorubicin. The area under a least squares fit of the ^{210}Po -citrate data to 2-component exponential function are shaded green. The green area *extends* completely under the red area, and therefore is larger than the red area. The red area corresponds to a least squares fit to the ^{210}Po -citrate+drug survival data *after* correction for drug toxicity (i.e. dotted curves in Figure 4). $\text{DMF} = (\text{green area})/(\text{red area})$.

FIGURE 6.

Representative Monte Carlo simulation of the fate of V79 cells based on agent uptake in individual cells after treatment with cocktails of ^{210}Po -citrate and daunomycin. Large bubbles represent survivors and small bubbles represent dead cells. **A)** 0.03 mBq/ cell ^{210}Po -citrate (0.1 mM citrate) + 0.63 μM daunomycin. **B)** 0.5 mBq/ cell (1.5 mM citrate) + 0.63 μM daunomycin **C)** 1.0 mBq/ cell (3.0 mM citrate) + 0.63 μM daunomycin. Note the decreasing number of survivors with increasing levels of ^{210}Po -citrate.

FIGURE 7.

Comparison of Monte Carlo simulated cell survival (symbols) with experimental clonogenic cell survival (solid curves) of V79 cells after treatment with combinations of (A) ^{210}Po -citrate + 0.63 μM daunomycin or (B) ^{210}Po -citrate + 2.50 μM doxorubicin. The experimental survival curves are least squares fits of the data presented in Figure 4. Open and closed symbols represent Monte Carlo simulations of cell survival when the agents are assumed to act independently and interactively, respectively. Error bars represent the SE for simulated surviving fraction based on fluorescence data from two independent experiments for each cocktail. Some error bars are smaller than the symbols.