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HIGH AND LOW LET PARTICLE-EMITTING ISOTOPES FOR THERAPY: A COMPARISON OF CYTOTOXICITY AND DOSE-RATE EFFECTS.

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Abstract

Radionuclides for therapy have been enhanced by the use of the monoclonal antibodies (MoAb). A major consideration when using low Linear Energy Transfer (LET) isotopes such as beta emitters is repair of sublethal damage when radiation is delivered at low dose-rates. Clinical studies of beta emitters linked to MoAbs to date have reported tumor dose rates estimated to range from 0.01 - 0.1 Gy/hour. Since cytotoxicity can be markedly reduced as the dose-rate decreases for low LET isotopes, we have examined Pb-212 and its high LET alpha-emitting daughter, Bi-212, which are less dose-rate dependent. They were complexed with DTPA and compared to Y-90 -DTPA in cytotoxicity studies with Chinese hamster cells. Clonogenic survival studies revealed no dose-rate effect for Pb-212 over a range of 0.1 - 0.6 Gy/h. When compared to Y-90, Pb-212 showed a 20-fold higher toxicity at the 10% survival level at a dose-rate of 0.6 Gy/h. The use of high LET radionuclides such as Pb-212 should be considered for radioimmunotherapy, particularly where radiation dose to the tumor is delivered at low dose rates.

Introduction

Targeted tumor therapy employing monoclonal antibodies (MoAb) and radionuclides may afford many distinct advantages in oncology. The most significant advantage centers about the ability of the MoAb to recognize and bind preferentially to the target cell of interest. Assuming that this specificity is large (between tumor and normal tissue) the choice of the appropriate radionuclide to attach to the MoAb is very important. There are presently a number of isotopes available for this purpose (1, 2).

The isotopes that have been used in clinical studies to date have predominantly been of low linear energy transfer (LET) with the primary mode of decay either photon and/or electron emission. Therapeutic tumor dose-rates thus far achievable with these isotopes has ranged from 0.01-0.1 Gy/hr (3). Such low dose rates for tumor treatment raises a serious radiobiological concern. For protracted dose rates within the dose rate range cited above, cells can repair radiation damage during the radiation exposure (4-6). This phenomenon, referred to as the dose-rate effect, applies for all low LET forms of radiation. As the dose-rate is lowered, cytotoxicity can be reduced markedly (4-6). In fact, for some cell types there is virtually no cell killing or modification in cell division for dose-rates below 0.1 Gy/hr (5).

These realities warrant evaluation of other isotopes of higher LET whose cytotoxicity is less dependent on dose-rate. The present study compares the cytotoxicity of a low versus

high LET radionuclide for low dose-rate exposure in solution. Pb-212 which decays through an alpha emitter to stability, was shown to be much more efficient in cell killing than Y-90, a beta emitter. No dose-rate effect was observed over the range of 0.1 to 0.6 G/hour for Pb-212 exposure, thus indicating that alpha emitters like Pb-212 may have utility in tumor radioimmunotherapy.

Materials and Methods

Exponentially growing cultures of Chinese hamster V79 cells were maintained in nutrient mixture F12 supplemented with 10% heat inactivated fetal bovine serum, penicillin, and streptomycin, at 37° C in a humidified, 5% CO₂/95% air atmosphere. For experiments where the cells were treated in monolayers, a number of T-25 flasks were seeded with 4×10^4 or 10^5 cells the day before the experiment. In experiments where the cells were treated in suspension, 10 ml of a 5×10^5 /ml cell suspensions was added to an appropriate number of test tubes (one tube per dose rate) immediately prior to the start of the experiment. Both the flasks and the tubes were gassed with 5% CO₂ in air after the addition of the radioactive isotope, and were sealed and incubated at 25°C and 37°C (monolayer) or 37°C with rocking to keep the cells suspended (suspension). All treatments were done in full medium. Controls consisted of the corresponding nonradioactive isotope attached to the chelate.

In the experiments utilizing Y-90 or Pb-212, the same amount of isotope was added to all flasks for a given dose rate, and different doses were delivered by incubating the cells with the isotope for differing amounts of time.

After the appropriate incubation times, monolayer cells were rinsed twice with PBS, trypsinized, resuspended in medium, counted and plated for macroscopic colony formations. Aliquots of cells from the suspension cultures were withdrawn at the appropriate times, diluted in PBS, centrifuged and rinsed with PBS, resuspended in medium, counted and plated for macroscopic colony formation. After 6-7 days of incubation, the colonies were fixed with methanol:acetic acid (3:1) and stained with crystal violet. Colonies of >50 cells were scored by eye and low power (10-40x) microscope. Surviving fraction was determined using the cold isotope control for each condition.

Radionuclide preparation: Pb-212 and Bi-212 were eluted from a Ra-224 generator (7). Both isotopes were eluted in equilibrium using 500 microliters of freshly prepared 2 N HI. Y-90 was purchased from Oak Ridge National Laboratory. The acid was neutralized to pH 4 with 1 N sodium acetate. 20 microliters of 10^{-3} M Na_3CaDTPA (Fluka) were added and the reaction mixed by vortexing.

After 10 minutes, the completeness of the reaction for the yttrium preparations was tested by spotting 5 microliters on a silica gel plate and eluting in a 1:1 mixture of methanol and 10% ammonium acetate (w/w). The R_f of the metal

complex was 0.6. Disodium phosphate was used to bring the pH of the solution to ~ 7. The radioactivity was assayed for the lead using a calibrated coaxial germanium detector interfaced with a multichannel analyzer. The 239 keV gamma ray was used to determine the lead activity. Y-90 dose was determined using a liquid scintillation counter.

Dosimetry calculations: The dose to the media was determined using the expression (8):

$$\frac{\text{g-Gy}}{\mu\text{Ci-hr}} = \frac{E_{\text{ave}} (\text{MeV}) \times 1.6 \times 10^{-6} \text{ ergs/MeV} \times \text{dis/hr}/\mu\text{Ci}}{100 \text{ ergs/g-rad} \times 100 \text{ rad/Gy}}$$

where E_{ave} is the average energy per disintegration of the atom.

Cell culture media was assumed to have unit density, therefore dose was calculated by dividing the dose from equation 1 by the volume of media in milliliters. In order to deliver given doses of radiation, the dose per microcurie was calculated and enough activity was added to deliver a given dose at the desired dose rate. The cells were spun down and washed twice with medium after the desired dose had been received by the cells. These cells were counted and plated for clonogenic assay.

Simultaneously, cells were counted either by gamma ray spectroscopy or by liquid scintillation counting to determine

the number of radioactive atoms bound to the cells after washing.

Results

Chinese hamster V79 cell survival following exposure to β^- particles from Y-90 in solution is compared with previous studies with external beam Cs-137 gamma rays at various dose rates is illustrated in Figure 1. It is clear from this figure that as the dose rate is decreased there is an enhancement in cell survival per unit dose. The survival curve generated by Y-90 irradiation at 0.6 Gy/hr falls in the range between 0.37 and 0.74 Gy/hr obtained from external Cs-137 gamma rays. This study serves to demonstrate that low LET radiation, whether it is delivered by secondary electrons from Cs-137 gamma rays or Y-90 β^- particles yields similar cell survival curves as the dose-rate is decreased.

The survival of V79 cells exposed to Y-90 and Pb-212 in solution at 0.6 Gy/hr is shown in Figure 2. Both survival curves were exponential with extrapolation numbers of 1.0. The D_0 for Pb-212 and Y-90 were 0.80 and 12.4 Gy, respectively. Pb-212 was clearly more efficient in cell killing. Based on a ratio of D_0 's, Pb-212 was found to be approximately 16 fold more effective in cell killing than Y-90. It should be pointed out that Pb-212 exposure at 0.6 Gy/hr. is also significantly more efficient in cell killing than Cs-137 exposure at high dose rates (1.43 Gy/minutes) as shown in Figure 1.

Cell survival was assessed for V79 cells for exposure to Pb-212 over the dose-rate range of 0.1-0.6 Gy/hr as shown in Figure 3. There was no increase in survival observed as the dose-rate was decreased. In fact, the lower dose rate exhibited slightly more cell killing. This was probably associated with the longer duration of Pb-212 exposure at the lower dose-rate and the binding of the Pb-212 to the cell surface. At the end of each exposure delivered at the 3 dose rates, cells were collected, rinsed, and counter for Pb-212 activity.

Cells exposed to 0.6 Gy/hr has essentially no activity associated with the cell pellet, whereas, for lower dose-rate exposures an average 1-2 atoms of Pb-212 were found to be bound per cell.

Discussion

Bi-212, an alpha emitting isotope, when attached to monoclonal antibodies was recently shown to be markedly more efficient in cell killing of human pancreatic carcinoma cells when compared to conventional x-rays (9). The results of the present study further emphasize the dramatic differences in cell survival achievable when cells are exposed to radiation of differing LET. Pb-212, the parent of Bi-212, was found to be 16 times more efficient in cell killing than Y-90 when compared at 0.60 Gy/hr. Additionally, the absence of a dose-rate effect for Pb-212 exposure highlights an important feature unique to high LET isotopes. Unlike low LET radiation, high LET radiation registers non-repairable cellular

damage independent of dose-rate (10, 11). The use of radiolabeled antibodies in cancer diagnosis is increasing and there is considerable interest in adapting this diagnostic tool for therapeutic purposes (12, 13). However, it must be appreciated that an isotope, while well suited for diagnosis, may not be the most beneficial selection for therapy. In general, the percent of injected dose that actually localizes in the tumor is quite small, typically 0.01 percent per gram or less (3). This reality poses two problems. First, the total dose delivered to the tumor is relatively low. Rarely does it approach the dose required for sterilization of a tumor. Second, the dose rate is low, usually ranging between 0.05 and 0.20 Gy/hr. Such dose-rates, particularly of low LET isotopes, would certainly not be considered as optimal therapeutically using conventional brachytherapy or implant standards. To further complicate matters, low LET isotopes presently used in treatment protocols have relatively short half lives, thus the dose rate is steadily decreasing once tumor localization has occurred. When dose rates are low (<0.1 Gy/hr.) cell survival following low LET radiation is greatly enhanced due to the repair of sublethal damage and actual cell division during the exposure (5). Thus, if low dose rate delivery of therapeutic radiation doses to the tumor is inevitable, use of high LET isotopes would seem logical, particularly for those tumors with efficient repair systems as these isotopes are efficient in cell killing at low dose rates. Maximal cell killing would be expected even when low tumor doses are delivered

due to the higher relative biological effectiveness of this form of radiation. Of course the use of low LET isotopes at low dose rate for those tumors with reduced radiation repair capacity would also serve the same purpose. Additionally, the combined use of drugs which might afford inhibition of radiation repair with low LET isotopes would be a way of achieving an improved therapeutic outcome.

The use of high LET isotopes for radioimmunotherapy poses some problems, however. The effective penetration of alpha emitting isotopes is but a few cell diameters. This would necessitate delivering multiple treatments to acquire adequate tumor dose to tumors of large size. Additionally, the half-life of Pb-212 is only 10 hours. This short half life would dictate the use of intracompartmental administration to maximize the access to the tumor while minimizing dilution in the blood pool and extravascular space. Normal tissue toxicity (particularly bone marrow) would potentially pose a problem as well for intravenous administration. Some of these problems apply regardless of the isotope selected as was recently pointed out (1). Radiation dose, dose-rate and isotope selection in relation to LET are definitely areas that must be addressed as the field of radioimmunotherapy progresses. The present study would support the consideration and evaluation of high LET isotopes, especially when repair proficient tumors are encountered clinically.

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Figure Legends

Figure 1. Survival curves for Chinese hamster V79 cells exposed to photons from an external Cs-137 source and Y-90-DTPA in the cell culture media. Dashed lines are from Mitchell, et al. 1979, (5) and the curve for Y-90 exposure was derived in the present study.

Figure 2. Survival curves for Chinese hamster V79 cells exposed to Pb-212 and Y-90 at 0.6 Gy/min. Multiple symbols represent replicate experiments.

Figure 3. Survival curves for Chinese hamster V79 cells exposed to Pb-212 at 0.1, 0.2, and 0.6 Gy/min.