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Neutron-Energy-Dependent Cell Survival and Oncogenic Transformation

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Both cell lethality and neoplastic transformation were assessed for C3H10Tl/2 cells exposed to neutrons with energies from 0.040 to 13.7 MeV. Monoenergetic neutrons with energies from 0.23 to 13.7 MeV and two neutron energy spectra with average energies of 0.040 and 0.070 MeV were produced with a Van de Graaff accelerator at the Radiological Research Accelerator Facility (RARAF) in the Center for Radiological Research of Columbia University. For determination of relative biological effectiveness (RBE), cells were exposed to 250 kVp X rays. With exposures to 250 kVp X rays, both cell survival and radiation-induced oncogenic transformation were curvilinear. Irradiation of cells with neutrons at all energies resulted in linear responses as a function of dose for both biological endpoints. Results indicate a complex relationship between RBE_m and neutron energy. For both survival and transformation, RBE_m was greatest for cells exposed to 0.35 MeV neutrons. RBE_m was significantly less at energies above or below 0.35 MeV. These results are consistent with microdosimetric expectation. These results are also compatible with current assessments of neutron radiation weighting factors for radiation protection purposes. Based on calculations of dose-averaged LET, 0.35 MeV neutrons have the greatest LET and therefore would be expected to be more biologically effective than neutrons of greater or lesser energies.

INTRODUCTION

In the United States during 1988 about 7,000 individuals per year in DOE facilities and about 6,000 research workers, well loggers, and reactor workers per year receive measurable neutron doses[1, 2]. In addition, airline crew members (300,000 in the US airline industry flying

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high-altitude routes) and astronauts in long manned space missions are expected to get significant neutron doses[3].

The neutron energy spectrum to which individuals will be exposed varies widely, depending on the neutron source and neutron moderation in shields including the body. Whether these effects are important depends on the variation in the relative biological effectiveness of neutrons over the neutron energy range of interest. The radiation weighting factors (w_R) currently recommended by the International Commission on Radiological Protection is 5 for neutrons of energy less than 10 keV, 10 for neutrons from 10 to 100 keV, and 20 for neutrons from 100 keV to 2 MeV[4,5]. The data on which the radiation weighting factors for low-energy neutrons are based are, however, quite limited.

To address the issue of the radiobiological effectiveness of neutrons, the Radiological Research Accelerator Facility (RARAF) Van de Graaff accelerator was used to expose C3H10T1/2 cells to essentially monoenergetic neutrons in the energy range 200 keV to 15 MeV. In addition, the accelerator was used to expose cells to two low-energy neutron beams with dose-averaged mean energies of 40 keV and 70 keV.

MATERIALS AND METHODS

Cell culture and preparation for irradiation

Exponentially growing mouse C3H10T1/2 clone 8 cells of fibroblast origin between passages 9 and 15 were used in these experiments. Cells were grown in Eagle's basal medium with 10% heat-inactivated calf serum supplemented with iron and 25 μg/ml gentamycin and cultured in a 37°C incubator with 5% CO_2-95% air.

For cell exposure to monoenergetic neutrons, cells were prepared for irradiation as described previously[6]. However, in order to minimize the dose and energy spreads of the two lowest energy neutrons, the procedure for exposing cells to either 40 or 70 keV neutrons was significantly different from cell preparations for higher energy neutrons. Twenty minutes before treatment, 2×10^5 cells concentrated into 0.2 ml of culture medium, purged with 5% CO_2-95% air, were added to 1ml pipettes. The opening of the pipette was sealed and cells were centrifuged at 1200 rpm for 5 minutes to pack cells towards the plastic plug of the pipette. Immediately after irradiation, cells were trypsinzed and replated into 100-mm-diameter tissue culture dishes at cell concentrations estimated to result in either 300 clonogenic cells per dish (for the transformation assay) or 30 clonogenic cells (for the cell-survival assay). At the end of the incubation period, cells were fixed in formalin and stained with Giemsa. Cell survival was determined by the colony assay method, and transformed foci types II and III were identified according to criteria described by Reznikoff et al[7,8] and the IARC/NCI/EPA Working Group[9].

Irradiation procedure

Monoenergetic neutrons with energies from 0.23 to 13.7 MeV were produced as described previously[6]. To produce low-energy neutron spectra, protons were accelerated towards a tritium target [^{3}H(p, n)^{3}He reaction]. Two different low-energy neutron spectra were used in this experi-

ment, with maximum neutron energies respectively of 110 keV and 65 keV. The dose-weighted average neutron energies for the two spectra were approximately 70 and 40 keV respectively.

RESULTS

Neutrons utilized for these experiments ranged from 0.23 to 13.7 MeV for monoenergetic neutrons and two low-energy neutron spectra with dose-weighted average neutron energies of 70 and 40 keV. Within each radiation modality, cell surviving fraction is clearly dose dependent. Figure 1 compares clonogenicity versus absorbed dose for the least biologically effective radiation (250 kVp) and the most effective neutron energy (0.35 MeV). All other neutron energies produced intermediate dose response curves. In general, as neutron energy increases biological effectiveness decreases.

Recognizing that spontaneous transformation frequencies fluctuate between experiments, Fig. 2 shows induced transformation frequencies per surviving cell as a function of absorbed dose for each neutron energy compared to 250 kVp X rays. For each individual experiment, separate control studies were performed, the resulting frequencies being subtracted from the transformation frequencies for that particular experiment. The error bars in Fig. 2 correspond to 95% confidence limits, assuming that the transformation frequencies conform to Poisson statistics as described previously[6].

Data for cell transformation after exposure to 0.040 and 0.070 MeV dose-averaged neutron energies are shown in Fig. 3. In addition, for comparative purposes transformation induction is

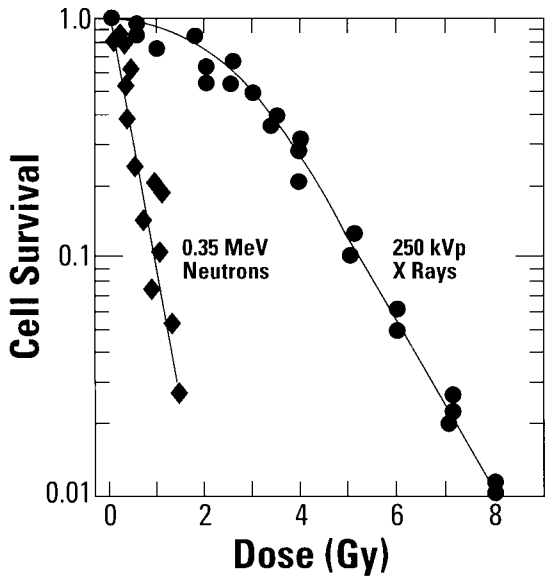

Fig. 1. Surviving fraction of mouse C3H10T1/2 cells following exposure to 250 kVp X rays and 0.35 MeV monoenergetic neutrons.

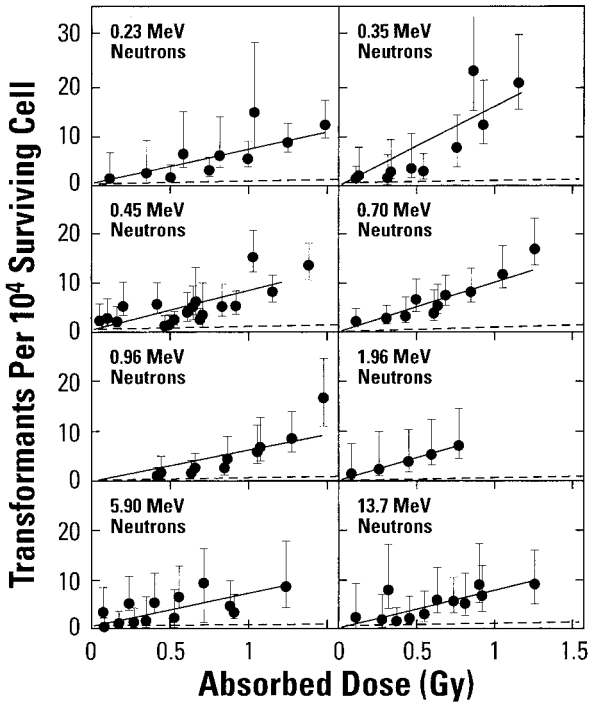

Fig. 2. Induction of oncogenic transformation per 10^4 surviving cells following irradiation with monoenergetic neutrons. Frequencies with error bars represent 95% confidence limits are shown. The dashed lines represent the fitted curve for 250 kVp X-ray exposures.

shown for cells exposed to 0.35 MeV monoenergetic neutrons and 250 kVp X rays. Induction of radiation-induced transformation for all neutron energies increases linearly with dose while a curvilinear response is seen for cells exposed to X rays. Analysis of the induction rates shows that the 0.040 MeV neutron beam was significantly less effective than 0.35 MeV neutrons (0.070 MeV neutrons were not different from 0.35 MeV neutrons).

The curvilinear response for X rays stands out relative to the near linear responses for each of the neutron energies. At limitingly low doses (where the quadratic term in a linear-quadratic model may be ignored), the maximal RBE becomes

$$\mathrm{RBE_m} = \alpha_n/\alpha_x, \tag{1}$$

In other words, the ratio of the initial slopes is determined. It should be stressed here that the quoted RBEs are model dependent.

Figure 4 shows the variation in $\mathrm{RBE_m}$ with neutron energy for the end points of survival and transformation. The general shape is comprised of an initial increase with neutron energy, followed by a decrease above a few hundred kiloelectron volts. This response is generally consistent with microdosimetric predictions, in that the neutron-induced recoil protons are shifted to lower

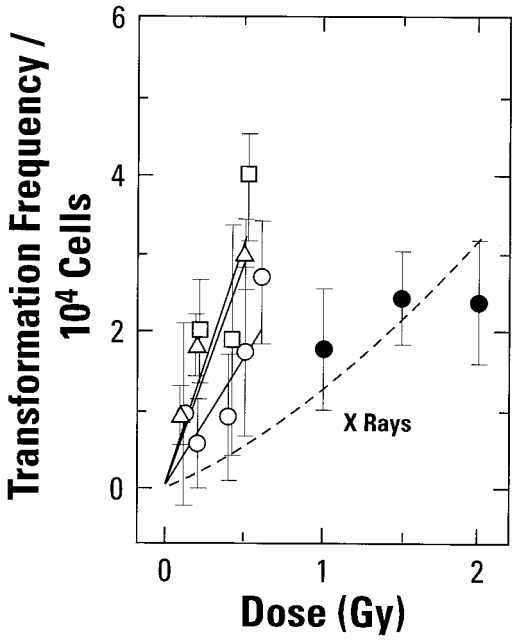

Fig. 3. Induction of oncogenic transformation per 10^4 surviving cells following irradiation with 0.35 MeV monoenergetic neutrons and two neutron energy spectra with average energies of 0.040 and 0.070 MeV. Frequencies with error bars represent 95% confidence limits are shown. The dashed lines represent the fitted curve for 250 kVp X-ray exposures.

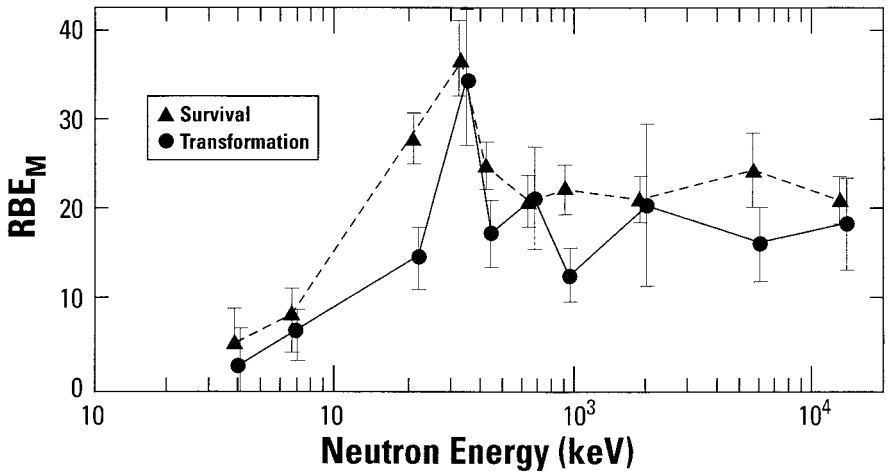

Fig. 4. Maximal relative biological effectiveness (RBE_m) for survival and transformation versus monoenergetic neutron energy. This represents the ratio of the initial slopes of the dose-response curves (α_n/α_x) for each energy. Error bars illustrate 95% confidence limits.

linear energies as the neutron energy increases, and the effect of heavy recoils is lessened by saturation effects.

DISCUSSION

It is interesting to note that the limiting RBE_m values for transformation and cell survival are not significantly different. A survey of the literature indicates that, in general, reported RBE values for transformation and survival tend to be similar. The same analysis techniques as described above for radiation-induced transformation and survival of C3H10T1/2 cells by Han and Elkind[10], yielded RBE_m values with Janus fission-spectrum neutrons of 14 ± 12 for survival and 20 ± 11 for transformation. Although the numbers are not directly comparable to those presented here (both the X ray and neutron-energy spectra were different), they do show a transformation RBE_m that is not significantly different from survival RBE_m values.

In radiation protection practice, the relative risks associated with exposure to low doses of various ionizing radiations are compared quantitatively by multiplying the absorbed dose with the radiation weighting factor (in the past this was known as the Quality Factor). Radiation weighting factor is evaluated based on biological experiments or from theoretical predictions when there is insufficient biological data.

Recently a variation of Quality Factor with neutron energy was proposed in ICRU Report 40[5]. This suggested a quality factor of 20 for the optimally effective neutron energy dropping to 10 for the least effective. Both the optimally effective neutron energy (about 0.35 MeV) and the variation with energy are consistent with the findings from this study.

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