Mutation Induction and RBE of Low Energy Neutrons in V79 Cells

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We have examined the neutron energy dependency of cell killing and mutation induction at the \textit{hprt} locus in Chinese hamster V79 cells. Monoenergetic neutrons at 0.32, 0.57, and 1.2 MeV were generated at the Hiroshima University Radiobiological Research Accelerator (HIRRAC) Facility, and were used to irradiate cells. The variation in RBE with neutron energy for the end points of cell survival and \textit{hprt} mutation induction was observed. When compared to $^{137}$Cs \textgamma-rays, all neutron energies were more effective at both cell killing and induction of mutation. Over the range of the neutron energies examined, we found that cytotoxicity increased as the energy decreased from 1.2 to 0.32 MeV. In comparison to \textgamma-rays, RBEs for cell lethality at 10\% survival were 5.7, 6.7, and 7.6 for 1.2, 0.57, and 0.32 MeV, respectively. Mutation induction, on the other hand, was highest at 0.57 MeV with a gradual decrease at 1.2 and 0.32 MeV. RBEs for mutation induction were 9.7, 19.4, and 13.9 for 1.2, 0.57, and 0.32 MeV neutrons.

We isolated independent V79 cell mutants at the \textit{hprt} locus from untreated and neutron-exposed cells and determined the genetic changes underlying the mutation by multiplex polymerase chain reaction (PCR)-based exon deletion analysis. Preliminary results are suggestive of a specific relationship between deletion patern and neutron energy.

INTRODUCTION

There has been a keen interest in the past decade in elucidating the biological effectiveness for different energy neutrons\textsuperscript{1–3). Risk assessment of individuals exposed to neutrons requires a knowledge of the RBE for a variety of end points including transformation and mutation with mammalian cells. However, very few of the studies provided data about the energy dependence of the effects of neutrons on mutagenesis\textsuperscript{4). In an earlier study, using fast neutrons produced by...
impinging protons with 46 to 14 MeV on to a beryllium target, a strong energy dependency was shown for neutron-induced mutation at both the \( hprt \) and \( tk \) loci in Chinese hamster V79 cells and human epithelial teratocarcinoma P3 cells\(^{5,6}\). However, further experimental studies are necessary to examine the energy dependency of neutrons with lower energies for mutation induction. To study the energy dependence of mutation induction after exposures to neutrons, we have examined mutation frequencies at the \( hprt \) locus after irradiation with low energy neutrons. Cells were exposed to neutrons with energies of 1.2, 0.57, and 0.32 MeV at the Hiroshima University Radiobiological Research Accelerator (HIRRAC) and \( \gamma \)-rays from a\(^{137}\)Cs irradiator.

The \( hprt \) gene codes for the purine salvage pathway enzyme hypoxanthine-guanine phosphoribosyl transferase. Analysis of mutations induced at the \( hprt \) locus is a well-characterized system used in the study of mammalian mutagenesis\(^7,8\). In the present paper, the mutant frequencies are presented for the \( hprt \) focus induced in V79 cells by neutrons. RBEs were calculated in the low dose region of the dose response curve with respect to \( ^{137}\)Cs \( \gamma \)-rays.

The greater effectiveness of neutrons in producing genetic changes is thought to be due to the different spatial distribution of damage. Independent \( hprt \) mutant clones were isolated only from experiments in which survival levels were 10 – 20% after irradiation with neutrons. We have examined the deletion spectra of all except 1 of the nine exons of the \( hprt \) locus in mutant V79 cells using multiplex polymerase chain reaction - based exon deletion analysis.

**MATERIALS AND METHODS**

**Cell culture**

The subline of V79-B310H of Chinese hamster lung fibroblasts originally described by Elkind and Sutton\(^9\) was used for this study. Cells were routinely subcultured in \( \alpha \)-MEM supplemented with 2.5% fetal calf serum and 7.5% new born calf serum, antibiotics and 20 mM Hepes. The cells were incubated at 37°C in a humidified atmosphere containing 2% \( \text{CO}_2 \).

**Radiation**

Monoenergetic neutrons were generated by \(^7\)Li(p,n)\(^7\)Be reaction at the Hiroshima University Radiobiological Research Accelerator (HIRRAC)\(^10\). Neutron energies used for the present experiments are 1.2, 0.57, and 0.32 MeV. Neutron doses were measured by paired (TE-TE and C-CO\(_2\)) ionization chambers. Contaminations of gamma rays were less than 6%. In parallel experiments, cells were irradiated with \(^{137}\)Cs \( \gamma \)-rays from Gamma Cell 40 at a dose rate of 1.1 Gy/min.

**Mutation**

For irradiation experiments, V79 cells were inoculated into 35-mm dishes at 5 \( \times \) 10\(^5\) per dish 24 h prior to the beginning of irradiation. After irradiations, cells were suspended by trypsinization, counted, and appropriate number of cells were plated in Petri dishes to measure cell survival or in large cell culture flasks for expression of mutants. At least 1 \( \times \) 10\(^6\) survival cells were initially seeded into flasks. For the survival assay, colonies were stained after growth.
of 7 days. Cells for mutation assay were reseeded in new large flasks on day 3 after irradiation so that exponential growth could be maintained for 6 days. On day 6, cells were trypsinized and 80,000 cells were plated per 100-mm Petri dishes containing 5 μg/ml 6-thioguanine to select for the \( hprt \)-deficient mutant cells. At the same time, 100 cells were inoculated in 60-mm dishes to determine the plating efficiency of the cells. Both colonies of plating efficiency and mutation frequency were scored after 7 days. The procedure described here is the standard procedure for \( hprt \) mutation assay and is outlined schematically in Fig. 1.

**Multiplex PCR-based deletion screening**

Multiplex PCR-based exon deletion screening was performed according to Xu et al\(^{11} \). Briefly, genomic DNA was isolated using a DNA Extractor WB Kit (Wako, Japan) from each mutant and wild type cells\(^{12} \). All exons, except 1, were amplified using seven pairs of oligonucleotide primers which flank seven fragments containing the \( hprt \) exons. Exon 7 and 8 were amplified in one fragment. PCR were performed in a capillary by Air Thermal Cycler (Idoho Technology) for 30 cycles (94°C, 0 s; 58°C, 0 s; 72°C, 15 s). PCR products were used for
RESULTS AND DISCUSSION

Cell killing and mutation frequency

Figure 2 shows the surviving fractions of V79 cells irradiated with neutrons from 1.2 to 0.32 MeV energies. Corresponding data for low-LET $\gamma$-rays showed a characteristic survival response with a shoulder and a Do value of 1.9 Gy. The survival curves for neutrons were approximated by an exponential function of dose. Cytotoxicity increased as the neutron energy decreased from 1.2 to 0.32 MeV. The calculated RBE values at the 10% surviving level were 5.7, 6.7, and 7.6 for 1.2 MeV, 0.57 MeV, and 7.6 MeV neutrons, respectively. Figure 3 shows RBE values as a function of neutron energy for cell lethality of V79 cells. Figure 3 also has RBE data from Zhu and Hill\(^5\) using neutron beams produced by impinging protons on a beryllium target at the UCLA/VA cyclotron. Neutrons from UCLA cyclotron have a wide range of energies up to a maximum of the proton energy. On the other hand, the neutron beams at HIRRAC are essentially monoenergetic. In this figure, we plotted the mean energy of neutrons from UCLA cyclotron. Both experiments were performed using the same clone of V79 cells and almost the same biological test system. Overall, the amount of lethal damage is dependent on the energy of the neutrons from 40 to 0.32 MeV.

Figure 4 shows the hprt gene mutation frequencies in V79 cells as a function of neutron doses for neutron energies ranging from 1.2 to 0.32 MeV. All neutron mutation curves appear to be linear at low doses. The data indicate that mutation frequency is maximal at 0.57 MeV neutrons. RBE values for mutation induction at a mutation frequency of 50 per million survivors

Fig. 2. The cell survival curves for V79 cells irradiated with monoenergetic neutron beams and $^{137}$Cs $\gamma$-rays. Error bars represent the standard errors of the mean for two or more experiments.
were 9.7, 19.4, and 13.9 for 1.2 MeV, 0.57 MeV, 0.32 MeV neutrons, respectively. Figure 5 shows RBE values as a function of neutron energies for mutation induction at the hprrt locus. Figure 5 also has RBE data of mutation induction using neutron beams from the UCLA/VA cyclotron. Overall, the induction of hprrt mutation is high at lower energies with a gradual decline with the increase of neutron energy. However, unlike RBE for cell killing, RBE for mutation induction is maximal at 0.57 MeV. These results suggest that the relationship of RBE values and neutron energies is dissimilar between the different types of biological end points.
Multiplex PCR for mutation analysis

We have adopted the multiplex PCR approach for the characterization of deletion mutation at the \( hprt \) locus. The molecular analyses of neutron induced \( hprt \) mutant clones are now an ongoing study, and only a limited number of clones has been examined so far. Early results looking at exon deletion by multiplex PCR are suggestive of a specific relationship between deletion pattern and neutron energies. Thus, preliminary result would imply that low energy neutrons appear to produce predominantly larger intragenic deletion and small or point mutations are relatively rare events.

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Fig. 5. RBEs as a function of neutron energies for mutation induction of V79 cells. Open circles are RBE for neutrons from UCLA cyclotron.\(^5\)
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