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EFFECT OF TRACK STRUCTURE AND RADIOPROTECTORS ON THE INDUCTION OF ONCOGENIC TRANSFORMATION IN MURINE FIBROBLASTS BY HEAVY IONS

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ABSTRACT

The oncogenic potential of high-energy ^{56}Fe particles (1 GeV/nucleon) accelerated with the Alternating Gradient Synchrotron at the Brookhaven National Laboratory was examined utilizing the mouse C3H 10T1/2 cell model. The dose-averaged LET for high-energy ^{56}Fe is estimated to be 143 keV/μm with the exposure conditions used in this study. For ^{56}Fe ions, the maximum relative biological effectiveness (RBE_{max}) values for cell survival and oncogenic transformation were 7.71 and 16.5 respectively. Compared to 150 keV/μm ^{4}He nuclei, high–energy ^{56}Fe nuclei were significantly less effective in cell killing and oncogenic induction. The prostaglandin E_1 analog misoprostol, an effective oncoprotector of C3H 10T1/2 cells exposed to X rays, was evaluated for its potential as a radioprotector of oncogenic transformation with high-energy ^{56}Fe. Exposure of cells to misoprostol did not alter ^{56}Fe cytotoxicity or the rate of ^{56}Fe-induced oncogenic transformation.

INTRODUCTION

The environment outside earth's magnetic shield is a complex mixture of high-energy protons, electrons, alpha particles and heavy ions. The short- and long-term health effects to astronauts exposed to ionizing radiation during extended space exploration are of paramount concern to NASA. The potential for significant exposure to high-LET particles from manned space missions raises concern for both stochastic effects (cancer induction and heritable disorders) and deterministic effects (primarily cataract induction).

Cytotoxicity, chromosome aberrations and oncogenic transformation induced by heavy ions have provided useful data for estimating the health risks from a variety of radiations (Yang *et al.*, 1985; Miller *et al.*, 1995; Wu *et al.*, 1997). Based on the risk estimates, consideration must be given to agents that could ameliorate the radiation health hazards inherent in manned space flights. Misoprostol, a prostaglandin E_1 analog (Cytotec, G. D. Searle) has been shown to be an effective cytoprotector and oncoprotector of cells exposed to X rays and fission-spectrum neutrons (Hanson *et al.*, 1988; Hanson and Grdina, 1991; LaNasa *et al.*, 1994).

In the present study, the potential of misoprostol to protect astronauts from inevitable space radiation was examined using C3H 10T1/2 cells exposed to high-velocity ^{56}Fe ions.

MATERIALS & METHODS

Cell Culture

The C3H 10T1/2 cell line has been used successfully for many years to quantify the cytotoxic and oncogenic potential of a variety of radiations with LET values ranging from 0.25 to over 600 keV/μm (Yang *et al.*, 1985; Miller *et al.*, 1995). This model system is quantitative and has generated results that are highly reproducible. Cells from passages 8-14 were used throughout the study and were cultured and assessed for transformed colonies as described (Miller *et al.*, 1995). Two days before exposure, cells were plated into culture flasks. Because of the nature of beam availability from the synchrotron at the Brookhaven National Laboratory (BNL), cells treated with misoprostol were exposed to the drug beginning 12 hr before irradiation and removed 2-3 hr post-irradiation at which time cultures were processed for survival and oncogenic transformation.

Misoprostol

Prostaglandins and leukotrienes are end products of the arachidonic acid metabolism cascade. Among their broad array of effects, prostaglandins have been shown to protect tissues from a variety of injurious agents (Hanson *et al.*, 1988; Hanson and Grdina, 1991). In addition, prostaglandins have been shown in several cell systems to be potent radioprotectors of cells exposed to X rays, gamma rays and fission-spectrum neutrons. In order to be maximally effective, cells must be treated with misoprostol for at least 2 hr before exposure to radiation. In preliminary studies, C3H 10T1/2 cells displayed a significant increase in survival when a 5μg/ml dose of misoprostol was administered for at least 2 hr before exposure to X rays.

Irradiation Sources

The Alternating Gradient Synchrotron (AGS) at BNL provided nominal 1 GeV/nucleon ^{56}Fe ions. Based on a comprehensive examination of the beam, Zeitlin *et al.* (1998) reported the dose-averaged LET with the irradiation setup that we used was 143 keV/μm. Exposure conditions including prolonged exposures to misoprostol were employed for cells exposed to 250 kVp X rays or high-energy ^{56}Fe. The source of X rays was a Westinghouse Coronado X-ray unit operated at 250 kVp, 15 mA with 0.25mm Cu and 1 mm Al external filtration that provided an output of 1.14 Gy/min. The Van de Graaff accelerator at the Radiological Research Accelerator Facility (RARAF) provided ^{4}He ions with an initial energy of 5.6 MeV (2.4 MeV in the cell) with a measured LET of 150 keV/μm. For the ^{4}He ions studies, cells were trypsinized from stock plastic culture dishes and plated at $2x10^5$ cells/dish onto thin Mylar-bottomed (6 μm thickness) stainless-steel dishes two days before irradiation. The Mylar dishes containing exponentially growing cells in monolayer were placed in a radiation wheel capable of holding up to 20 dishes. Immediately after irradiation, cells were removed from the Mylar surface and processed for examination of cell survival and oncogenic transformation as described in the previous section on cell culture techniques.

RESULTS

Dose response survival curves to 250 kVp X rays, 150 keV/μm ^{4}He nuclei and 1 GeV/nucleon ^{56}Fe are shown in Figure 1. Cells exposed to X rays display an initial shallow slope (α) followed by a continuously bending curve at higher doses (β). In contrast, cells exposed to either 150 keV/μm ^{4}He ions or 1 GeV/nucleon ^{56}Fe exhibit a dose response relationship where survival is a simple exponential function of dose. If the initial slopes are compared ($\alpha_{\text{He nuclei}}/\alpha_{\text{X rays}}$ and $\alpha_{\text{Fe-56}}/\alpha_{\text{X rays}}$), the RBE_{max} values for survival are 15.1 and 7.71 respectively.

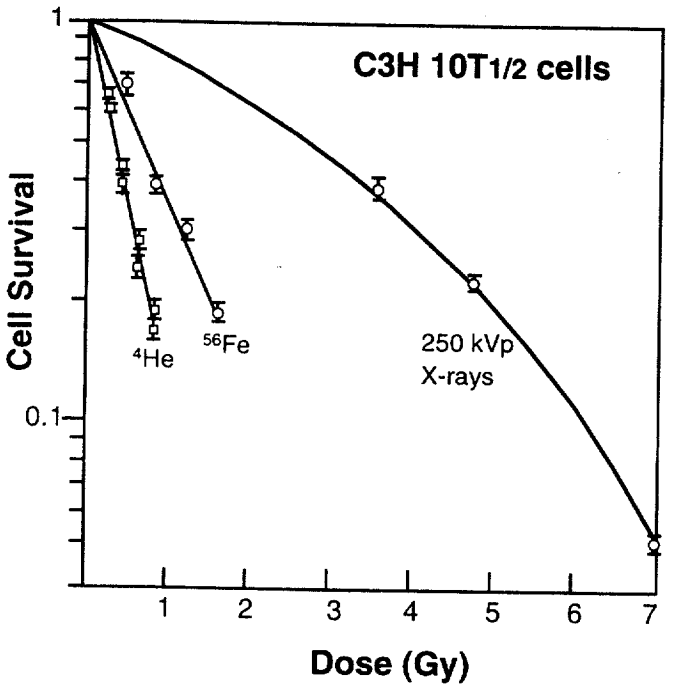

Fig. 1. Cell survival for C3H 10T1/2 cells exposed to X rays, high energy ^{56}Fe ions and ^{4}He ions. Error bars represent ±1 standard deviation.

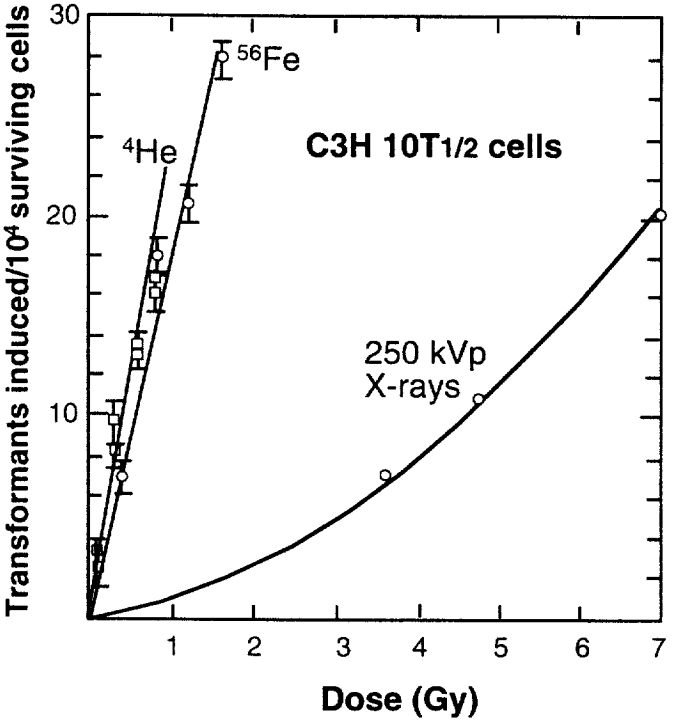

Fig. 2. Oncogenic transformation for C3H 10T1/2 cells exposed to X rays, ^{56}Fe ions and ^{4}He ions. Error bars represent ±1 standard deviation.

Oncogenic transformation for C3H 10T1/2 cells exposed to 250 kVp X rays, 150 keV/μm ^{4}He nuclei and 1 GeV/nucleon ^{56}Fe are shown in Figure 2. Data for direct comparison of the radiation types were fitted to the equation, $R_i = b + \alpha_i D + \beta D^2$ using a single common value of α for both radiations. In this equation, b is the background transformation rate, D is dose and subscript i refers to the radiation type. For each pair of radiation types, the residuals from the individual-α and the common-α fits were then compared using an F test, to examine the null hypothesis that the data from both radiation types came from the same distribution. In other words, instead of evaluating the significance of individual points, all data points were fit to a common α and residual errors (goodness of fit) were examined. It is apparent by this comprehensive method that oncogenic induction is quite different between the qualities of radiation. X-ray induction is curvilinear while ^{4}He and ^{56}Fe inductions are essentially linear. Comparison of the initial slopes for transformation induction results in RBE_{max} values of 22 and 16.5 respectively. In this series of experiments, the spontaneous transformation frequency was 0.65 x 10^{-4} transformants per surviving cell.

Clonogenic survival for C3H 10T1/2 cells exposed to 1 GeV/nucleon ^{56}Fe and X rays in the presence or absence of misoprostol was also measured. Misoprostol showed significant protection of cells exposed to X rays with a dose-reduction factor (DRF) of 1.2. However, misoprostol showed no ability to protect cells exposed to 1 GeV/nucleon ^{56}Fe nuclei (data not shown).

CONCLUSIONS

Extended manned space flights inevitably will result in significant doses to astronauts from high-LET radiation that includes high-energy ^{56}Fe nuclei. In this series of experiments, we examined the oncogenic potential of high-energy ^{56}Fe and compared heavy ion transformation induction with 250 kVp X rays and 150 keV/μm ^{4}He nuclei. Selection of 150 keV/μm ^{4}He nuclei is based on microdosimetric calculations that indicate the LET for 1 GeV/nucleon ^{56}Fe is ~143 keV/μm. However, it is already known that LET is not a good indicator of biological effect for heavy particles. Based on these studies, there is a significant difference for both cell lethality and oncogenic transformation between the two particles of similar LET. Indeed, several other investigators have made similar observations with cell survival and chromosomal aberrations as biological endpoints (Goodhead *et al.*, 1992; Goodwin *et al.*, 1996; Durante *et al.*, 1998).

Although exposure may be unavoidable, the biological effects from deep space exposures may be modified. Studies have demonstrated the ability of misoprostol to protect a wide variety of tissues from the toxic and oncogenic effects of X and gamma rays (Hanson *et al.*, 1988). In addition, misoprostol has recently been shown to protect tissue from damage after exposure to fission-spectrum neutrons (Hanson and Grdina, 1991). We conclude from the present study that misoprostol is neither a cytoprotector nor an oncoprotector of cells exposed to high-energy ^{56}Fe ions. Although the mechanism of action of misoprostol is not fully known, some of its cytoprotective qualities may result from its ability to stimulate repair of radiation damage. Since exposure to high-LET radiation significantly reduces the cell's ability to repair complex damage, it would seem that misoprostol was unable to restore full repair function. However, the value of misoprostol as a radioprotector remains since studies by Hanson and Grdina (1991) have demonstrated that with high-LET fission-spectrum neutrons, the combination of amifostine (administered as WR1065) and misoprostol showed significant radioprotection compared to either agent alone.

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