

Micronuclei induction in human fibroblasts exposed in vitro to Los Alamos high-energy neutrons

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Abstract

High-energy secondary neutrons, produced by the interaction of galactic cosmic rays with the atmosphere, spacecraft structure and planetary surfaces, contribute to a significant fraction to the dose equivalent in crew members and passengers during commercial aviation travel, and astronauts in space missions. The Los Alamos Nuclear Science Center (LANSCE) neutron facility's ICE House 30L beamline is known to generate neutrons that simulate the secondary neutron spectra of earth's atmosphere. The neutron spectrum is also similar to that measured onboard spacecraft like the MIR and International Space Station (ISS). To evaluate the biological damage, we exposed human fibroblasts in vitro to the LANSCE neutron beams without degrader at an entrance dose rate of 25 mGy/h and analyzed the micronuclei (MN) induction. The cells were also placed behind a 9.9 cm water column to study the effect of shielding in the protection of neutron induced damages. It was found that the dose response in the MN frequency was linear for the samples with and without shielding and the slope of the MN yield behind the shielding was reduced by a factor of 3.5. Compared to the MN induction in human fibroblasts exposed to a γ source at a similar low dose rate, the RBE was found to be 16.7 and 10.0 for the neutrons without and with the 9.9 cm water shielding, respectively.

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1. Introduction

Galactic cosmic, solar particle and trapped particle radiation produce secondary radiation in the form of high energy neutrons by interacting with the atmosphere, spacecraft structure and planetary surfaces (Wilson et al., 2004). These high energy neutrons can be a significant contributor to the absorbed dose and dose equivalent received in crew members and passengers during commercial aviation travel and astronauts in space missions (Lindborg et al., 2004;

Getselev et al., 2004). Inside the Space Station, secondary neutrons may account for as high as 30% of dose equivalent (Badhwar et al., 2000).

The biological effect of neutrons varies widely as a function of the neutron energy. The International Commission on Radiological Protection (ICRP) has recommended the weighting factor for neutrons to be in the range of 5–20 depending upon the energy and with the peak weighting factor of 20 for energies in the range of 0.1–2 MeV (ICRP, 1990). The weighting factor was proposed to be a continuous function of neutron energy in a later published report by ICRP with a lower factor for thermal neutrons (ICRP, 2003). The relative biological effectiveness (RBE) of neutrons also varies for different biological endpoints and different cell types. For instance, the RBE for oncogenic transformation of

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C3H10T1/2 cells for 70 keV fission neutrons was estimated to be 6.6 (Miller et al., 2000), while the RBE for chromosome aberrations in human lymphocytes exposed to fission neutrons of the same energy was found to be 53 (Edwards et al., 1982).

While most of the reported investigations of neutron-induced biological impacts are for fission spectrum neutrons, few studies have been done on neutrons of broad energy spectrum that characterize the environment in the upper atmosphere and in space. The energy spectrum of the LANSCE ICE House 30L beamline is well known to have characteristics that simulate the secondary neutron spectra of earth's atmosphere. It has been shown that the spectrum is also similar to that measured onboard spacecraft like the MIR and International Space Station (ISS) (Badhwar et al., 2000). These qualities make the 30L beamline uniquely suited to radiation experiments relevant to aerospace applications, including radiation biology experiments on secondary neutron effects.

In this study, human fibroblasts were exposed, *in vitro*, to the ICE House 30L high energy neutron spectrum at a low absorbed dose rate. The samples were subsequently analyzed using cytokinesis-blocked micronucleus (MN) assay to determine the level of biological damage. The MN assay is one of the most commonly used methods for measuring DNA damage rates in human populations and has been successfully applied in many ground-based space radiation studies. A significant increase in MN induction was observed in tracheal and lung epithelial cells from Wistar rats irradiated with HZE as compared to those exposed to low-LET γ radiation (Brook et al., 2001). Additionally, structured energy deposition from the tracks of primary protons and the associated high-LET secondary particles produced in targets has been attributed to the DNA damage differences, as measured by MN (Oliveira et al., 2003). It is also well known that both low- and high-LET irradiation induce bystander effects as measured by MN in non-hit cells (Shao et al., 2004). Recently, we have reported MN inductions across the Bragg curve of energetic charged particles (Wu et al., 2006; Desai et al., 2006). Similar to other biological endpoints, the RBE for neutron-induced MN yield could vary from 12.2 for human lymphocytes (Huber et al., 1994) to 216 for dry dormant seeds (Zhang et al., 2003). Although individual variations in the background MN frequency limits the use of the assay to measure genetic damage in human populations, the MN assay is commonly used to detect early radiation damages (Bonassi et al., 2007).

2. Materials and methods

2.1. Radiation and neutron measurement

The neutron energy spectrum at the ICE House target area was generated by an 800 MeV pulsed proton beam that strikes a tungsten target causing spallation neutrons

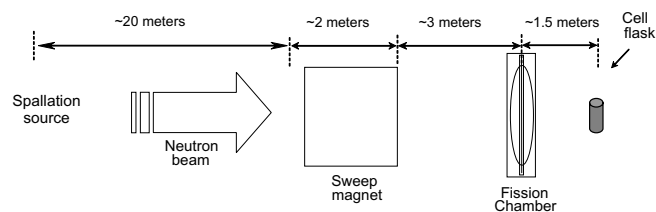


Fig. 1. Experimental setup of exposure of cultured cells to secondary neutrons at LANSCE.

to be generated (Lisowski et al., 1990). A sweep magnet upstream of the ICE House target area removes charged particles generated during the spallation process from the beam. The absolute neutron intensities in the energy range from 1 MeV to 800 MeV were measured by a fission chamber and time of flight (TOF) system (Wender et al., 1993). Fig. 1 illustrates the setup used in this series of experiments.

The dose rate of neutrons at the entrance was measured to be 25 mGy/h. The dosimetry information for this study was measured using a Tissue Equivalent Proportional Counter (TEPC) that was functionally identical to the TEPC used onboard the space shuttle. For neutron exposures, one flask of cells filled with medium was placed at the entrance and one placed behind a 7.4 cm water shield. The dose rate behind the total of 9.9 cm water shielding (entrance flask plus water shield) was measured to be 11.8 mGy/h. Similar exposure setup was used for the exposure to γ -rays without shielding at NASA Johnson Space Center (JSC). The dose rate of γ -exposure at the location of the sample was 17 mGy/h.

2.2. Cell culture and micronuclei assay

Normal human foreskin fibroblasts (AG1522, National Institute of Aging) of low passages were routinely cultured (Nalgene) at 37 °C, with 95% humidity and 5% CO₂, in α -MEM containing 10% fetal calf serum. Confluent cells in T-25 flasks were filled with medium, sealed and kept in a heater/cooler at 37 °C during the exposure period that lasted up to 10 h for the highest dose. The neutrons passed through a thin plastic door before hitting the flasks. The cells were subsequently subcultured in slide chamber flasks at low density in the growth medium containing 2.5 μ g/ml cytochalasin B.

A cytokinesis blocking technique was performed according to the method of Fenech and Morley (1986). Briefly, after incubation in cytochalasin B for 48 h, cells were washed with PBS and fixed with methanol/acetic acid (3:1, vol/vol) for 15 min. For micronuclei analysis, the cell nuclei were stained with DAPI (Wu et al., 2006), and MN were scored in binucleated cells and classified according to the standard criteria (Fenech et al., 2003). Approximately 1000 binucleated cells were scored for each dose point for both neutron and gamma irradiated samples.

3. Results and discussion

Fig. 2 shows the measured energy spectrum of neutrons before and after the water shield at the location of the cells. The spectrum of neutrons in the atmosphere at 12,000 m altitude (Hewitt et al., 1978), when multiplied by 3×10^5 , resembles closely the measured spectrum at Los Alamos in the energy range of 1–1000 MeV. The water shield appears to scatter the neutrons of energies below 100 MeV. As expected, neutrons of energies above 100 MeV were less affected by the shielding material. The average quality factor of the neutrons measured with and without the water shield was found to be 17.4 and 12.5, respectively, using the ICRP (1990) recommended quality factors. Badhwar et al. (2000) reported the average quality factor to be 16.9, which was close to the current value. The same authors (Badhwar et al., 2000) also reported an average quality factor of 14.9 after a 5 g/cm² polyethylene degrader containing 13% ¹⁰B (PE/B-10) and of 10.3 after a 20 g/cm² shield of the same material. Our average quality factor of 12.5 after a 9.9 cm water shield falls in between these two values, as expected. In the present study, the dose rate after the 9.9 cm water shield was measured to be 47% of the entrance value and the dose equivalent was reduced by a factor of 3.0 after the shield. The effect of shielding in the induction of chromosome aberrations for charged particles has also been investigated (Durante et al., 2005).

The MN yield with and without the water shield was presented in Fig. 3 as a function of dose measured only in front of the shield. The slope of MN induction was $2.3 \pm 0.1 \text{ Gy}^{-1}$ and $0.66 \pm 0.06 \text{ Gy}^{-1}$ before and after the 9.9 cm water shield, respectively, resulting in a reduction of 3.5 times with the shielding. This value is close to the reduction of the average quality factor of 3.0 from the present physical measurement. Badhwar et al. (2000) reported that the dose equivalent was reduced by a factor of 3 after 20 g/cm² degraders of two polyethylene materials, but the

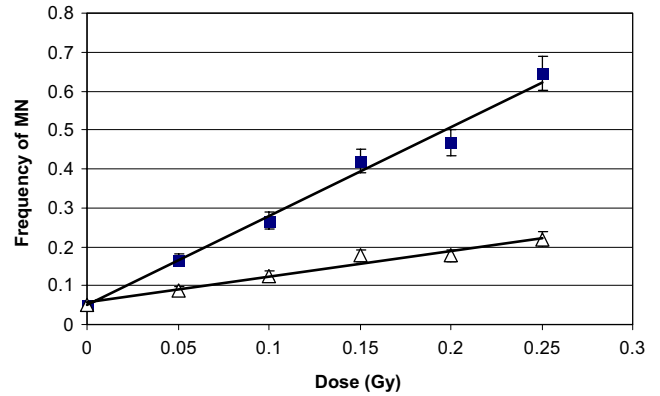


Fig. 3. The frequency of MN before (solid square) and after (open triangle) a 9.9 cm water shield. The dose is the entrance dose only. The slope of the fitted linear line is found to be 2.3 ± 0.1 and $0.66 \pm 0.06 \text{ Gy}^{-1}$ for yield before and after the shield, respectively. Therefore, the 9.9 cm water shield reduced the induction of MN by a factor of 3.5.

dose equivalent after 5 g/cm² degraders of various materials was actually greater than that at the entrance. Although the present results suggest that sufficient shielding does reduce the biological damages from the neutrons found in the upper atmosphere and in space, further investigations will be conducted to study effectiveness of thin shields.

To evaluate the RBE of the neutrons before and after the water shield, we plot in Fig. 4 the frequencies of neutron-induced MN as a function of dose measured at the sample location, together with MN induced by low dose rate γ rays. The dose response for all curves can be fitted nicely by a linear line, and the slope of γ ray-induced MN was found to be $0.14 \pm 0.01 \text{ Gy}^{-1}$. Thus, the RBE value for the entrance neutrons was 16.4 ± 1.4 . This RBE value is similar to that of 12.2 for MN induction in human lymphocytes exposed in vitro to fission neutrons (Huber et al., 1994), which appears to the closest comparison avail-

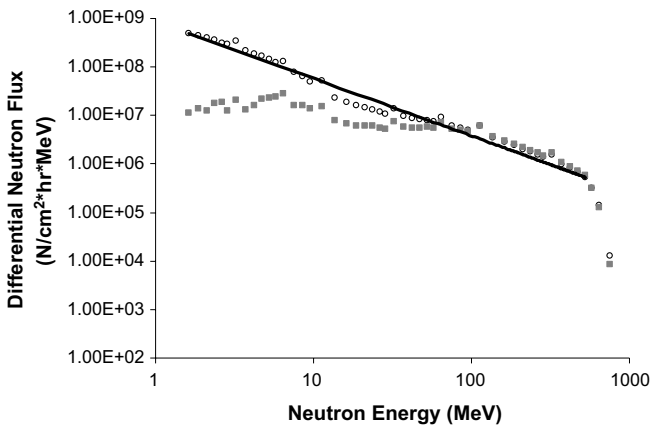


Fig. 2. The energy spectrum of secondary neutrons generated at the 30L beamline at LANSCE before (open circle) and after a 9.9 cm (solid square) water shield. The shape of the spectrum without the shield is similar to that in the upper atmosphere multiplied by 3×10^5 as represented by the solid line.

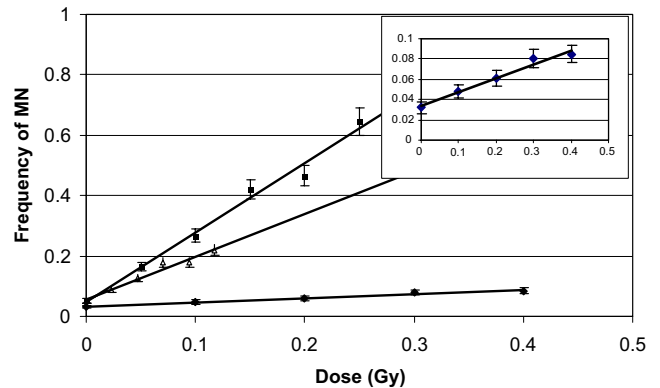


Fig. 4. The dose response of MN induced by neutrons before (solid square) and after (open triangle) a 9.9 cm water shield, and by low dose rate γ rays (solid diamond). The dose was measured at the sample location. The RBE was 16.4 ± 1.4 and 10.0 ± 1.0 for neutron before and after the shield, respectively. The inset is the dose response for γ rays in a smaller y-scale.

able. However, for chromosome aberrations in human lymphocytes which is tightly related to MN, the RBE was reported to be in the range between 53 for 0.7 MeV fission neutrons and 30 for 7.6 MeV cyclotron neutrons (Edwards et al., 1982). Although MN is a different biological endpoint, it is interesting to note that the present RBE value is close to the average quality factor measured with physical instruments.

Behind the 9.9 cm water shield, the slope of MN induction shown in Fig. 4 was $1.4 \pm 0.1 \text{ Gy}^{-1}$, resulting in a RBE of 10.0 ± 1.0 . Although a comprehensive data set of MN as a function of mono-energetic neutrons is unavailable, for dicentric formation, it has been shown that the RBE value decreased as the energy of neutrons increased beyond 0.5 MeV (Schmid et al., 2003; ICRP, 1990). The change of RBE in the present study is apparently a consequence of the loss of low energy neutrons below 100 MeV after the shielding, as shown in Fig. 2. It should be noted that the RBE obtained in the present study is significantly higher than the RBE of fission neutrons of MN formation in mouse reticulocytes in vivo (Kagawa et al., 2004).

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