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SCIENCE DIRECT®
Advances in Space Research 35 (2005) 243–248
ADVANCES IN SPACE RESEARCH (a COSPAR publication)
www.elsevier.com/locate/asr

DNA DSB induced by iron ions in human fibroblasts: LET dependence and shielding efficiency

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Received 26 July 2004; received in revised form 22 October 2004; accepted 19 November 2004

Abstract

This paper reports on DNA DSB induction in human fibroblasts by iron ions of different energies, namely 5, 1 GeV/u, 414 and 115 MeV/u, in absence or presence of different shields (PMMA, Al and Pb). Measure of DNA DSB was performed by calibrated Pulsed Field Gel Electrophoresis using the fragment counting method.

The RBE–LET relationships for unshielded and shielded beams were obtained both in terms of dose average LET and of track average LET. Weak dependence on these parameters was observed for DSB induction.

The shielding efficiency, evaluated by the ratio between the cross sections for unshielded and shielded beams, depends not only on the shield type and thickness, but also on the beam energy. Protection is only observed at high iron ions energy, especially at 5 GeV/u, where PMMA shield gives higher protection compared to Al or Pb shields of the same thickness expressed in g/cm^2.

Keywords: Space radiation; HZE particles; DNA DSB; PFGE; RBE–LET relationship; Radiation shielding

1. Introduction

Galactic Cosmic Rays represent one of the main sources of radiation outside the magnetic field of the Earth. They include high charge and energy (HZE) particles that are of special concern for their potential effects on the astronaut's health during long term space flights. Risk reduction can be provided by effective radiation shielding inside the spacecraft (Wilson et al., 1995, 1997). As a consequence of their interaction with a shield, HZE particles fragment and deposit energy at rates depending on their type and energy, and on the nature and thickness of the shield. The biological effectiveness of the mixed field emerging from the shield needs to be properly understood in order to evaluate the shielding effectiveness. It is out of the question, for HZE particles, to consider the possibility to have a shield of reasonable weight which is sufficient to stop the beam. Thus the primary particles, that have not changed their identity in nuclear interactions, emerge from the shield with lower energy. If this were the only effect, then the shield would worse things, since decelerated particles will generally have higher LET and greater biological effectiveness. Therefore, the beneficial consequences of a shield will have to derive from the nuclear production of secondary particles, which will change not only the energy, but also, in a substantial way, the charge composition of the particles reaching the cellular targets. The need then arises to build a shield such that the emerging composite beam will be really less efficient

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0273-1177/$30
doi:10.1016/j.asr.2004.11.021

in damage induction. It is not obvious a priori that this will be the case with any shield; then studies are necessary to characterize the different situations.

To this purpose an international collaboration was started some years ago aimed at studying the influence of the shielding on biological effectiveness of HZE particles for several cellular end points. In this framework ground-based experiments have been performed using the accelerators at the Brookhaven National Laboratory (BNL), USA, and at the National Institute for Radiological Sciences (NIRS), Japan.

Although it is widely recognised that the spatial distribution of DNA damage depends on the radiation quality and that correlated DSB are produced by the same particle track (Goodhead, 1994; Holley and Chatterjee, 1996), to our knowledge there are no biological data concerning the effects of shielding on DSB induced by HZE particles.

In this paper we present data on DNA double strand breaks (DSB) induction in human fibroblasts exposed to accelerated iron ions in the absence and in the presence of polymethylmethacrylate (PMMA), aluminium and lead shields.

2. Materials and methods

2.1. Cell culture and sample preparation

The normal human fibroblasts AG1522 cell line was obtained from the National Institute of Aging (NIA) cell repository (Coriell Institute for Medical Research, USA). Cells were grown as monolayer in alpha-Minimum Essential Medium, containing 1 mM glutamine, supplemented with 20% foetal calf serum, 2% Hepes buffer solution (1 M), 50 U/dm^3 of penicillin and streptomycin (all reagents from Gibco).

Cells were cultured for at least five generations to reach and maintain confluence in the presence of 1.85×10^3 Bq/cm^3 ^{14}C-thymidine (NUNC). The labeled medium was substituted with unlabeled one 24 h before irradiation. Cells were then detached by addition of trypsin–EDTA, pooled, centrifuged and resuspended at the final concentration of about 1.3×10^6 cells cm^{-3} in 0.8% (w/v) low-gelling agarose (Sigma Type VII) made up in PBSS/EDTA buffer. A volume of 85 μl of this suspension was pipetted into $7 \times 5 \times 2$ mm moulds (Bio-Rad) and allowed to form plugs at 4 °C.

2.2. Irradiation

AG1522 cells embedded in agarose plugs were irradiated with Fe ions of various energies. Irradiation with 5 and 1 GeV/u Fe ions was performed at the Alternate Gradient Synchrotron (AGS) and at the NASA Space Radiation Laboratory (NSRL) facilities of the Brookhaven National Laboratory (BNL), Upton, USA. Irradiation with 414 and 115 MeV/u Fe ions was performed at the Heavy Ions Medical Accelerator (HIMAC) of the National Institute for Radiological Sciences (NIRS), Chiba, Japan. γ-rays from a ^{60}Co source were used at the Istituto Superiore di Sanità, Rome, Italy, as reference radiation.

Irradiations with 5 and 1 GeV/u and 414 MeV/u were also performed in the presence of different shields, namely PMMA, Al and Pb.

In order to avoid DSB repair during irradiation, plug holders that allow to maintain the temperature at 0–4 °C were especially designed to fit the beam geometry at the two facilities.

Doses up to about 250 Gy were delivered to the samples. Dose rates of ≈10–15 Gy/min were used for unshielded beams. After shielding the dose rate changed by factors ranging from 0.27 to 1.75, depending on the beam energy and on the type and the thickness of the shield.

Beam characteristics under the various irradiation conditions are reported in Table 1 (Durante et al., in press; Miller and La Tessa, personal communications). The track-average LET (L_T) and the dose-average LET (L_D) listed in the table represent the first and the second moment of the LET distribution, respectively, so that their knowledge gives the expected value and the width of this distribution, being its variance, in particular, given by $L_T \cdot (L_D - L_T)$. It can be noted that for the unshielded 5 GeV/u Fe beam the difference between L_D and L_T reflects a significant contamination of lighter ions, mainly protons, as shown by measurements with Si detectors (Miller, personal communication).

Dosimetry was performed by ionization chambers at BNL (Zeitlin et al., 1998) and by ionization chamber and CR39 plastic nuclear track detectors at NIRS (Durante et al., 2002; Grossi et al., 2004).

Shield insertion changes the dose rate measured at the sample position. The ratio between the dose incident on the shield and that at the sample position (in the same time interval) is defined here as the "dose rate reduction factor" (DRRF) and listed in the last column of Table 1.

The dose per unit fluence for the unshielded beams can be evaluated from the track-average LET (L_T): $D/F = 0.16 L_T/\rho$, where D is the dose in Gy, F is the fluence in μm^{-2}, L_T is in keV/μm, and the value $\rho = 1$ g/cm^3 was used. For the shielded beams, this value divided by the dose reduction factor gives the dose at sample position per unit fluence of particle impinging on the shield.

2.3. DSB measurements

After irradiation, the plugs were carefully removed from the moulds and incubated in lysis solution (0.5