DOES HZE RADIATION-INDUCED DNA DAMAGE DIFFER FROM LOW-ENERGY HIGH LET RADIATION DAMAGE?

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INTRODUCTION

It is important to verify that DNA damage produced by HZE ions is similar to damage produced by lower energy high LET ions with consideration to the track structure, which in the case of HZE ions involves high-energy delta rays. Such comparisons will help rule out the suspicion that HZE radiation, due to its unique properties, could act with different unusual mechanisms.

METHODS

We have used 1 GeV/u and 600 MeV/u iron ions at NSRL, 300 kVp X-rays and 25 MeV/u nitrogen ions from the 88” cyclotron at Lawrence Berkeley National Laboratory. For measurements of DNA double-strand breaks we are using pulsed-field gel electrophoresis (PFGE) with a dual label technique that includes control samples in each lane and the use of the most sensitive part of the FAR vs dose curve. This methodology allows us to use the same low doses for DSB measurements (0.2 – 2 Gy) as is used for the PCC damage measurements and micronuclei formation. Immuno-staining and counting of [H2AX] foci is used as an alternative method to score DSBs. PCC is induced by calyculin-A, and scored in coded slides. Chromatid and iso-chromatid breaks in PCC samples are scored in G2/M cells. Bystander effects are measured in cells that receive medium from irradiated cultures (medium transfer technique).

RESULTS

With our sensitive pulsed-field gel electrophoresis method we have compared DSB induction in human and rodent cells using 1 GeV/u Fe-ions, 25 MeV/u N-ions, and 300 kVp X-rays in the dose range of 0.2 –2 Gy. We find that the fraction of activity released (FAR) for the 1 GeV/u Fe ions falls between the N-ion data and the X-ray data. The latter result is what would be expected based on track structure considerations. We have also compared DSB and prematurely condensed G2/M chromatid break yields and found both N-ions and Fe-ions yield 5-fold lower levels of PCC breaks compared to DSBs. This supports the hypothesis that the mechanism for generation of this type of cytogenetic damage is similar for the two ions.

To investigate repair of DSBs after low doses of Fe-ions, N-ions and X-rays we are measuring the formation of phosphorylated H2AX ([H2AX]), which occurs in the chromatin surrounding DSBs. In addition to counting [H2AX] foci by microscopy, we are using a FACS-based assay to measure cellular levels of [H2AX] in the G1, S and G2 phases of the cell cycle. We have looked at [H2AX] levels remaining 18 hr after 1 Gy of Fe-ion radiation and found approximately 10% of the signal remains. This is comparable to published values of DSBs remaining for other types of high LET radiation.

The above results do not hint of anything special about HZE particle radiation. However, we have also compared micronuclei formation in hamster cells after exposure to 1 GeV/u Fe-ions and 600 MeV/u Fe-ions. Our expectation from track structure considerations was that the 600 MeV/u ions would be slightly more efficient than the 1 GeV/u ions. Surprisingly, we found instead a remarkable higher yield of micronuclei formation after 1 GeV/u ion radiation. This result, if confirmed, may point to the possibility of some unknown deleterious effect as the energy of the ion is increased.