J. RADIAT. RES., 43: SUPPL., S149–S152 (2002)

Clustered DNA Damages Induced in Human Hematopoietic Cells by Low Doses of Ionizing Radiation[a]

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DNA damage / Clusters / Mononuclear cells

Ionizing radiation induces clusters of DNA damages—oxidized bases, abasic sites and strand breaks—on opposing strands within a few helical turns. Such damages have been postulated to be difficult to repair, as are double strand breaks (one type of cluster). We have shown that low doses of low and high linear energy transfer (LET) radiation induce such damage clusters in human cells. In human cells, DSB are about 30% of the total of complex damages, and the levels of DSBs and oxidized pyrimidine clusters are similar. The dose responses for cluster induction in cells can be described by a linear relationship, implying that even low doses of ionizing radiation can produce clustered damages. Studies are in progress to determine whether clusters can be produced by mechanisms other than ionizing radiation, as well as the levels of various cluster types formed by low and high LET radiation.

INTRODUCTION

Damage by space radiation to cells of the human hematopoietic system could pose major health risks to travelers to the International Space Station, or on the Mars Mission. However, little is known of the effects of radiation on human primary hematopoietic cells. A critical radiation-induced damage is thought to be complex bistranded clusters, composed of multiple lesions on opposing DNA strands within a few helical turns[1–4]. Such clusters could be highly detrimental to cells either because their attempted repair could generate additional double strand breaks[5,6], or because clusters are difficult to repair and thus would comprise persistent damages. As a first step in evaluating the effects of clustered damages in primary human hematopoietic cells, we have asked if clustered damages could be quantified in the same dose range as significant biological effects on such cells. Our data show that clusters can readily be measured in a biologically-significant range, that their induction can be described by a linear dose-response, and that DSBs comprise a minority among radiation-induced complex damages.

MATERIALS AND METHODS

Human monocytes (28SC, American Type Culture Collection CRL-9855) were grown on Iscoves' modified Dul-

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[a] Research supported by a grant from the National Space Biomedical Institute (IIH00207) to AG and BMS, by a National Institutes Health grant (R01 CA86987) to BMS, by the Office of Biological and Environmental Research of the US Department of Energy, and by CNRS and Electricité de France, Contrat Radioprotection,

[b] This research was reviewed and approved by the Institutional Review Boards for human studies of The University of Pennsylvania (06/19/01) and Brookhaven National Laboratory (6/19/01).

becco's medium (Gibco/BRL, Grand Island NY), supplemented with 10 % fetal bovine serum (Hyclone, Logan UT) without antibiotics as previously described[7]. They were ascertained to be free of mycoplasma by periodic testing (Bionique, Saranac Lake NY). Cells were chilled on ice, then exposed while on ice to 0–1 Gy of 50 kVp X-rays at a rate of 0.7 Gy/min. Cells were harvested immediately after irradiation by immersion in liquid nitrogen.

DNA was isolated by Proteinase K digestion of cells in agarose plugs, then cleaved with NotI in agarose plugs with minimum exposure to air[8]. One plug from each sample was incubated with buffer alone, while the companion plug was treated with sufficient homogeneous Nth protein to cleave at all substrate sites[9–17]. Clusters recognized by *E. coli* Nth protein are Nth-OxyPyr clusters.

Samples were electrophoresed along with molecular length standard DNAs (*Saccharomyces cerevisiae* chromosomes, ladders) using neutral transverse alternating field electrophoresis[18]. Gels were stained with ethidium bromide, destained, and a quantitative electronic image obtained[19]. A DNA dispersion curve was determined, the number average lengths of the experimental DNAs were determined, and from them, the frequencies of DSBs and clusters were calculated[7,20,21].

RESULTS

Bistranded clustered damages are measured using the approach shown in Figure 1. Human cells are unirradiated (controls) (See Panel A), or exposed to radiation, inducing complex damages and isolated lesions (Panel B). These damages are recognized by *E. coli* Nth protein (Endonuclease III), whose principal substrates are oxidized pyrimidines. This enzyme releases the oxidized base by its glycosylase function, and makes a single strand break at each site with its lyase activity (Panel F). For Nth-OxyPyrimidine clusters, complex damages composed of an oxidized pyrimidine (or other Nth protein substrate) *vs.* another closely spaced oxidized pyrimidine, oxidized abasic site or single strand break on the opposite strand, Nth protein treatment produces a *de novo* double strand break (in addition to those produced directly by radiation), at each Nth-OxyPyrimidine cluster site, thus reducing the (double stranded) DNA size. Nth protein action on isolated lesions produces single strand breaks, which do not reduce the size of the DNA double strands (Panel F). The unirradiated DNA, whether or not treated with Nth protein, remains as high molecular length molecules (Panels C and D). An electronic image of a neu-

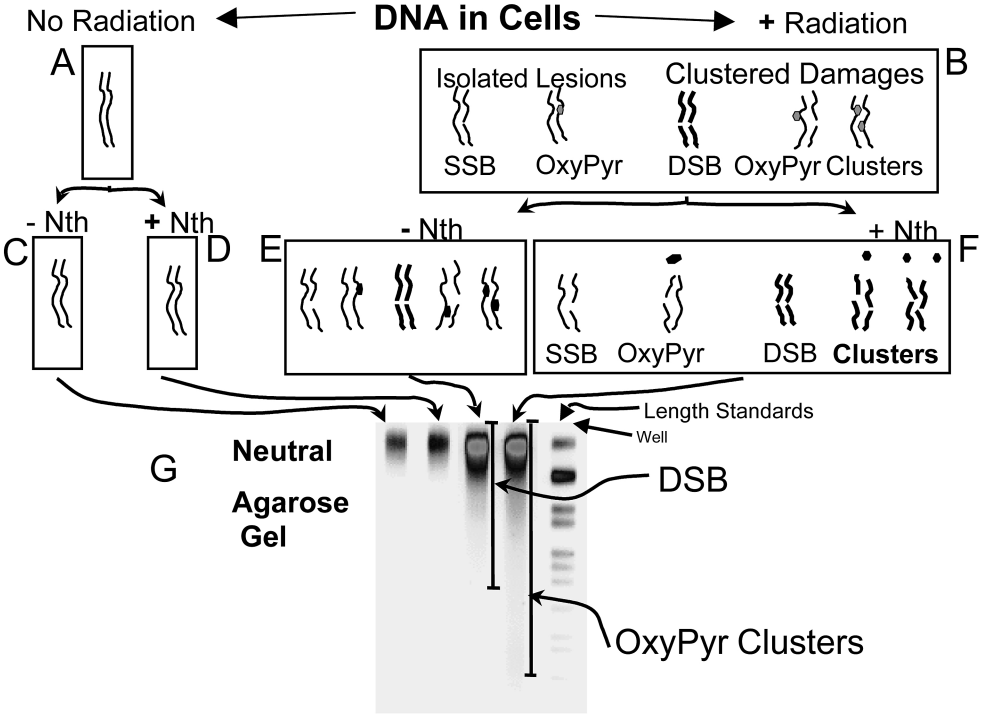

Fig. 1. Principles of analysis of bistranded clustered damages using pulsed field gel electrophoresis and number average length analysis, illustrated here for DSBs and oxidized pyrimidine clusters (recognized by cleavage with Nth protein).

tral pulsed-field electrophoretic gel containing such samples is shown in Panel G, with length standard DNAs and four experimental lanes. The frequency of clusters is calculated by number average length analysis of sample DNAs.

We used this method to quantify specific bistranded clustered damages in human cells irradiated with 50 kVp X-rays. Figure 2 shows the relative yields of Nth-Oxidized Pyrimidine Bistranded Clusters induced by X-rays (Panel A)[8] and by Fe particles (1 GeV/amu) (Panel B). The figure shows that the induction responses can be fit reasonably by straight lines (fit by a least squares fitting procedures; fitting parameters 0.98 and 0.96 for X-ray and Fe data, respectively). Clearly the yields of such complex damages can be readily detected in the range of 0–100 cGy. We have also measured the effect of low LET radiation on hematopoietic progenitor cells derived from human bone marrow, and have shown that the ability of these cells to form mature lineages in vitro is significantly affected by doses in this range (data not shown). This suggests that it will be possible to measure cluster induction and repair in such cells in a biologically-relevant dose range.

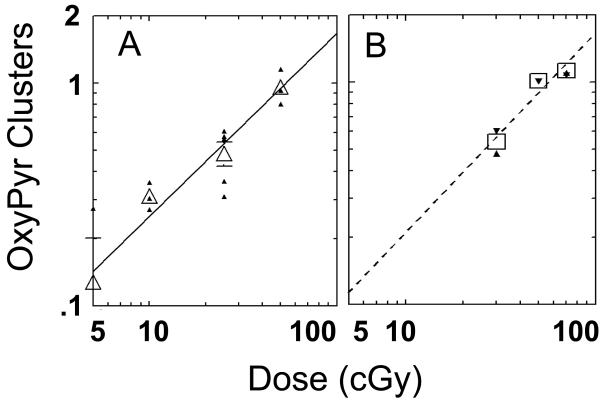

Fig. 2. Frequencies of bistranded oxidized pyrimidine clusters produced by 50 kVp X-rays (A) or by Fe particles (1 GeV/amu), (B). Values are normalized to those of DSBs at 50 cGy. Small symbols, individual data points; large open symbols, averages.

DISCUSSION

Although unrepaired double strand breaks are well-known to be lethal and mutagenic (leading to DNA rearrangements, including chromosomal aberrations) damages, the consequences of other complex damages is not known. Their constituent lesions have been associated with induction of mutations[22]. It is thus critical to know the levels induced by low and high LET radiation, as well as the biological consequences of such complex damages. The data show that both X-rays (50 kVp) and Fe particles (1 GeV/amu) induce oxidized pyrimidine clusters in DNA in human cells, and that the levels are rather similar on a per dose basis.

Evaluating the radiation risk to space travelers requires being able to measure their induction and repair or attempted repair — including the role of sequence context[23]— in tissues, essential to human health, such as cells of the hematopoietic system. Previous studies have examined cytogenetic alterations in such cells[24] and studied effects on the whole animal[25], but nothing is know of cluster induction and repair in these cells. One way of approaching this project is beginning at the molecular level, then investigating biological effects at the cellular, tissue and whole organism levels. We have taken a first step in this undertaking by asking if we could measure clustered damages in the same dose range as the primary human hematopoietic cells. We find that OxyPyrimidine clusters can readily be measured in the dose range of 0–100 cGy, the same dose range in which the primary mononuclear cells show significant killing by radiation. Such studies will provide a molecular foundation for future investigations of the possible importance of complex damages in humans during space travel.

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Received on May 14, 2002
Revision on October 4, 2002
Accepted on November 1, 2002