1st International Workshop on Space Radiation Research and
11th Annual NASA Space Radiation Health Investigators' Workshop
Arona (Italy), May 27-31, 2000

DNA fragmentation induced in K562 cells by nitrogen ions

M. Belli[1,3], V. Dini[1], G. Esposito[1,3], P. Micera[1], O. Sapora[2,3], C. Signoretti[1,3], G. Simone[1,3], B. Stenerlöw[4], M.A. Tabocchini[1,3]

1. *Physics Laboratory, Istituto Superiore di Sanità, Rome (Italy)*
2. *Comparative Toxicology Laboratory, Istituto Superiore di Sanità, Rome (Italy)*
3. *INFN - Gr. coll. Sanità, Rome (Italy)*
4. *Department of Oncology, Radiology and Clinical Immunology, Uppsala University, Uppsala (Sweden)*

Abstract

This study was aimed at investigating the radiation induced DNA fragmentation pattern as a function of cellular differentiation and radiation quality.

DNA double strand breaks (DSB) induced by γ-rays were analyzed in K562 human proerithroblasts before (AP cells) and after (D cells) differentiation induction while DNA DSB induced by 125 keV/μm N-ions have been studied in AP cells. Pulsed-Field Gel Electrophoresis (PFGE) of cellular DNA was used to determine the DSB yield by analysis of the Fraction of Activity Released (FAR) and of the fragmentation pattern in a specific size range (5.7-0.225 Mbp).

The results so far obtained show that the DSB induction by γ-rays is different if evaluated with the FAR or with the fragmentation analysis. The DSB yield obtained with the former method is about 1.4 times higher in AP respect to D cells while the latter method indicates that more fragments are produced in D cells. Comparison between γ-rays and N-ions in AP cells shows that no significant differences are detected by the FAR analysis; otherwise fragmentation analysis demonstrates a higher effectiveness of nitrogen ions.

KEYWORDS: Cellular differentiation, DNA DSB, FAR analysis, fragmentation analysis.

1. Introduction

The K562 erythroblastoid cell line [1] represents a well-known cellular system capable to undergo butyric acid-induced pseudoerythroid differentiation. It can be used for evaluating whether the radiation damage is affected by the structural and metabolic changes accompanying the differentiation process. In particular, the chromatin structure has been reported to be more condensed in differentiated than in proliferating cells, with larger loops anchored to the nuclear membrane [2, 3]. These features can be of great relevance in determining the production and distribution of DNA damage, especially DSB.

Radiation quality is another important factor affecting DNA damage. The high effectiveness of densely ionizing radiation in producing cellular effects has been related to the induction of severe DNA lesions induced in higher proportion respect to sparsely ionizing radiation. It is expected that the primary ionization sites be produced by densely ionizing radiation in a correlated manner along the particle track. This can lead to the occurrence of clustered or associated lesions that produce a DNA fragmentation pattern different from that produced by sparsely ionizing radiation [4].

In the present paper preliminary results on radiation induced DNA fragmentation as a function of cellular differentiation and radiation quality are reported.

2. Materials and Methods

2.1. *Cell culture and differentiation*

K562 cells were grown as suspension culture in humidified 5% CO_2 atmosphere at 37°C in RPMI-1640 medium supplemented with antibiotics, L-glutamine and FCS, with a doubling time of about 24h. DNA was labeled by growing the cells in the presence of 1.85 kBq cm^{-3} [^{14}C]-thymidine for 72h. Cellular differentiation was induced incubating the cells in the presence of 1-2 mmol dm^{-3} butyric acid.

2.2. *Irradiation*

Actively proliferating (AP) cells and cells at 72h from differentiation induction (D) were irradiated on ice with γ-rays from a ^{60}Co source at the Istituto Superiore di Sanità. AP cells were also irradiated at +4°C with 125 keV/μm (LET_∞) N-ions at the Gustaf Werner synchrocyclotron of the Bio-Medical Unit, The Svedberg Laboratory, Uppsala [5].

2.3. *Electrophoresis conditions*

After irradiation, the cells were centrifuged and resuspended in low-gelling agarose (0.7% w/v in PBSS-EDTA buffer). Plugs containing 1×10^6 cells

were then incubated in lysis solution (0.5 mol dm^{-3} EDTA pH 8.0, 1% sarkosyl, 1.0 mg cm^{-3} proteinase K) for 1h at +4°C followed by overnight incubation at +50°C. DNA damage was evaluated by calibrated Pulsed Field Gel Electrophoresis (PFGE) performed using a CHEF DRIII system (BioRad) following the conditions reported in [6] for separation of DNA fragments in the size range 5.7-0.225 Mbp. After electrophoresis, gels were stained with ethidium bromide for 2h at +4°C and visualized on an UV transilluminator. Each lane of the gel was cut in slices of about 3 mm in correspondence of specific size markers. Each slice was put into scintillation vials and hydrolyzed with HCl at +70°C over night. After addition of scintillation cocktail (ULTIMA GOLD-XR, Packard), the radioactivity in each sample was measured using a Wallac Beta Counter.

2.4. *Calculation of DNA DSB*

The number of induced DSB was calculated on the same data sets by two different methods.

i) Evaluation of the overall FAR out of the well performed using the *random breakage* model derived from Cook and Mortimer [7]:

$$FAR = F_{max}\{1 - [1 + (N_{DSB} - N_{DSB,0})\,K(1 - K/M_0)]\,\exp[(N_{DSB} - N_{DSB,0})K]\} \quad (1)$$

where: N_{DSB} and $N_{DSB,0}$ are the number of D_{DSB} in the irradiated and in the control sample, respectively; F_{max} the fraction of cellular DNA effectively entering the gel; M_0 the average chromosome size (assumed to be 180 Mbp); K the exclusion size (taken as the larger marker size clearly separated from the compression zone, i.e. 5.7 Mbp).

ii) Direct calculation of the number of fragments (practically equal to the number of DSB) performed according to the method used by Loebrich and coworkers [8]:

$$n(\overline{M_i}) = F_i / \overline{M_i} \quad (2)$$

where $n(\overline{M_i})$ is the number of DNA fragments of average size $\overline{M_i}$ induced per unit DNA mass; F_i is the fraction of DNA mass in the slice i of the gel. $\overline{M_i}$ was approximated by the size corresponding to the average between two consecutive DNA markers.

3. Results and Discussion

DNA fragments revealed under the PFGE conditions described above are of size comparable with the loop dimension. Therefore, the fragmentation profiles so obtained can be related to the interplay between the radiation track structure and chromatin organization at the loop level.

Concerning the two different types of analysis performed to evaluate DSB, the FAR analysis is based on a random breakage model, while the fragmentation analysis does not require any specific assumption for the breakage mechanism.

Figure 1 (panel A and B) shows the FAR values plotted as a function of dose for the different cellular and irradiation conditions examined. The DNA DSB yield for γ-rays was calculated by fitting the data with equation 1, assuming a linear relationship between the total number of induced DSB and the dose d (N_{DSB} = y d M_0). This yield is about 1.4 times higher in AP respect to D cells [$y_{AP,\gamma} = (5.35 \pm 0.44)\ 10^{-9}\ bp^{-1}\ Gy^{-1}$ and $y_{D,\gamma} = (3.74 \pm 0.10)\ 10^{-9}\ bp^{-1}\ Gy^{-1}$]. No significant differences are observed changing radiation quality in AP cells.

The results obtained by fragmentation analysis in the size range 5.7-0.225 Mbp are also shown in the figure (panel C and D). In this case, the number of DSB after γ-irradiation is higher in D with respect to AP cells. In AP cells N-ions appear more effective than γ-rays.

The non-linearity of the dose-response evaluated by fragment counting can be ascribed to the limited size range analyzed. In contrast, FAR analysis considers the entire fragmentation spectrum below the exclusion size, assumed to be given by random breakage mechanism. However, the last method has been shown to underestimate DSB, especially for heavy ions [8].

Our finding that at the same FAR values the N-ions induce more fragments than γ-rays indicates that the two radiations induce different DSB distribution in cellular DNA. In order to better exploit the fragmentation method, evaluation of fragments distribution in a lower molecular weight range is under investigation.

Acknowledgements

This work was partly supported by the EC Contract FIGH-CT-1999-0012.

REFERENCES

[1] Lozzio CB, Lozzio BB. Human chronic myelogenous leukemia cell-line with positive Philadelphia chromosome. Blood 1975: 45; 321-334.

[2] Rothman C, Cohen AM, Malik Z. Chromatin condensation in erythropoiesis resolved by multipixel spectral imaging: differentiation versus apoptosis. J Histochem Cytochem 1997: 45; 1097-1108.

[3] Hartwig M, Matthes E, Kopp J, Jantscheff P, Millek J. DNA organization changes after butyrate-induced differentiation of leukemic K562 cells. Studia Biophys 1984: 99; 185-192.

[4] Goodhead DT. Initial events in the cellular effects of ionizing radiation: clustered damage in the DNA. Int J Radiat Biol 1994: 63; 7-17.

[5] Stenerlöw B, Blomquist E, Grusell E, Hartman T, Carlsson J. Rejoining of DNA double-strand breaks induced by accelerated nitrogen ions. Int J Radiat Biol 1996: 70; 413-420.

[6] Newmann HC, Prise KM, Folkard M, Michael BD. DNA double-strand break distributions in X-ray and α-parti-

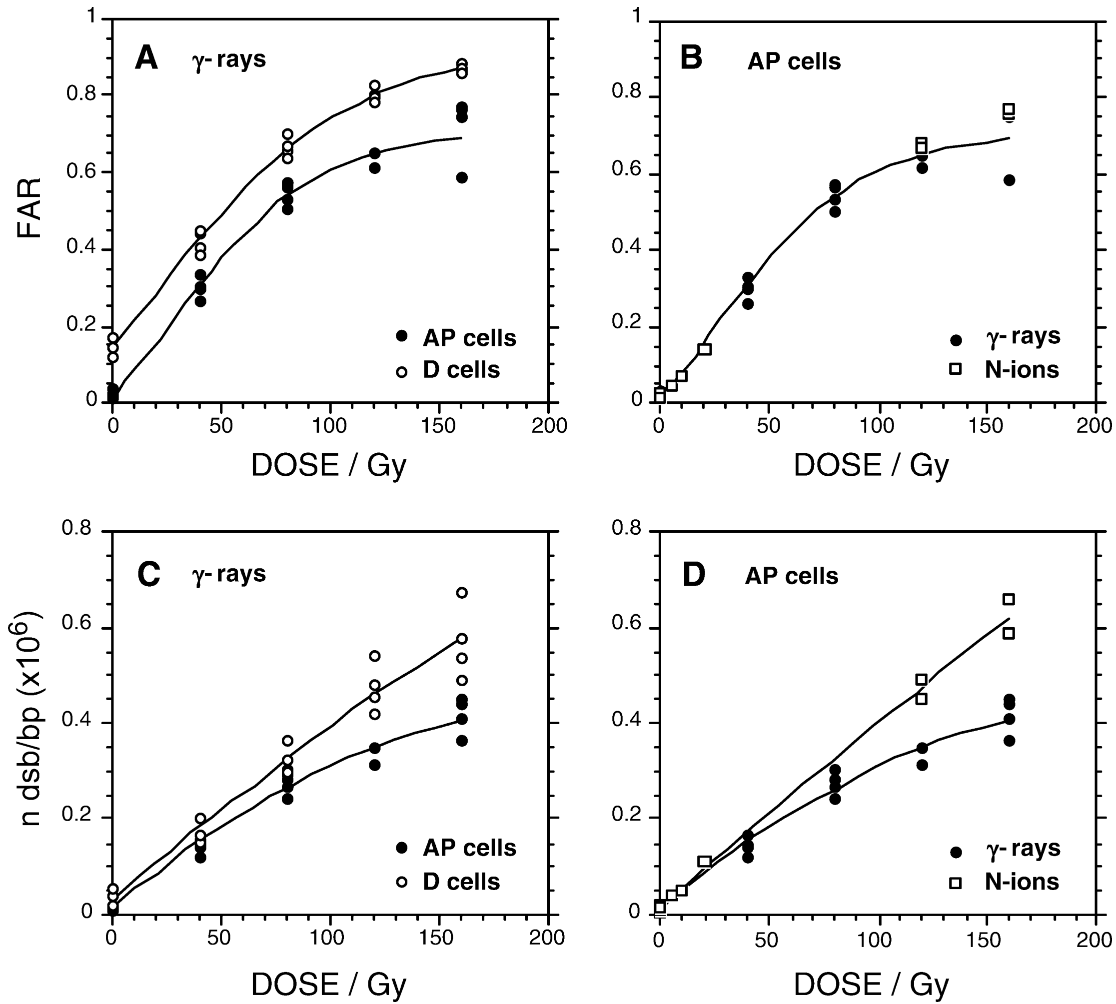

Fig. 1 – Fraction of ^{14}C-labeled DNA released from the plug (FAR) vs dose (panel A and B) and number of DSB vs dose (panel C and D). (●) K562 actively proliferating cells (AP) irradiated with γ-rays or (□) 125 keV/μm N-ions; (O) K562 cells at 72h from differentiation induction (D) irradiated with γ-rays. Lines in panel A and B represent the best fits of the experimental data obtained using Eq.1 (see Materials and Methods); lines in panel C and D only represent guide for the eye.

cles irradiated V79 cells: evidence for non-random breakage. Int J Radiat Biol 1997: 71; 347-363.

[7] Cook VE, Mortimer RK. A quantitative model of DNA fragments generated by ionizing radiation and possible experimental applications. Radiat Res 1991: 125; 102-106.

[8] Loebrich M, Cooper PK, Rydberg B. Non random distributions of DNA double-strand breaks induced by particle irradiation. Int J Radiat Biol 1996: 70; 493-503.