CELL DEATH AND DNA LESION CAUSED BY ACCELERATED CHARGED PARTICLES

K. Eguchi-Kasai, T. Kosaka, I. Kaneko, T. Takahashi, A. Maruhashi* and K. Nakano
Riken, Wako-shi, Saitama 351-01, and *Univ. Tsukuba, Sakuramura, Ibaraki 305, Japan

Summary. DNA double strand break (dsb) in cultured human and chinese hamster cells was studied after irradiation with γ-rays and particle beams. Non-repairable dsb in both cell lines qudratically increased with dose of ^{60}Co γ-rays and linearly with dose of particle beams. These curves were in order of radiation sensitivity for cell killing. This result strongly suggests the close relationship between non-repairable dsb and cell killing by radiation.

Introduction

It is important to study biological effect of charged particle radiations because charged particles are useful for cancer therapy, space radiation safety and radiation biology. Mammalian cells are sensitive to charged particle beams than γ-rays or X-rays. The absorbed dose required to reduce the cell survival fraction to 37% (D_{37}) is in the range of 2 to 5 Gy of ^{60}Co γ-rays and about 1 Gy of charged particles. The dose-survival curve (natural log of surviving fraction is plotted against radiation dose) for charged particles is nearly linear whereas that for γ-rays is convex. Ritter et al. insisted that non-repairable DNA breaks closely related to the cell death induced by charged particles because these breaks were induced by a function of LET similar to mammalian cell inactivation.[1] In that work, however, since extremly higher doses (up to 450 Gy) of heavy ions than upper limit of the dose to examine survival (up to 15 Gy) were used to detect DNA breaks, the results cannot be directly related to the cell death. Recent work showed the similar LET-RBE relationship of DNA breaks using low doses (20 Gy).[2] But these works did not explain the difference in the shape of dose-survival curves and did not show even the nature of non-repairable DNA breaks.

We have already showed that N-ion beam induced single strand breaks and DNA-protein crosslinks much more than X-rays.[3] Here we examined the DNA double strand breaks (dsb) in 2 mammalian cell lines with different radiosensitivity caused by ^{60}Co γ-rays, α-particles and N-ions in comparison with cell killing.

Materials and Method

Cells

We used 2 cell lines with different radiosensitivity, HMV-I cells derived from human melanoma (tumor cells) and HA-1 cells derived from chinese hamster cells ("normal" cells). The cells were cultured in growth medium supplemented with 10% fetal bovine serum and antibiotics at 37°C in a humidified atmosphere of 5% CO_2 in air.

Table 1. List of radiations.

cells	radiation	LET (keV/μm)
HMV-I	α-particles	77
	N-ions	530
HA-1	α-particles	36

Irradiation

Nitrogen-ion and α-particle (0.8-2 Gy/min) from RIKEN cyclotron and ^{60}Co γ-rays (1.4 Gy/min) were used. Irradiation procedure for particle beams was previously described.[4] Beam currents were monitored by a Faraday cup[4] and transmission ionizing chamber during irradiation. The stopping poweres of the particle beams were listed in Table 1. Cell were irradiated at room temperature in most case except to test dsb induced immediately after irradiation with ^{60}Co γ-rays, where cells were irradiated on ice.

Detection of dsb

Neutral (dsb) elution technique which were very sensitive to detect breaks[6] was used. Relative number of dsb per unit amount of DNA (No. dsb) was calculated as follows:

$$\text{No. dsb} = -\log F$$

where F was the DNA fraction remaining on a filter after 4 h elution. No. dsb was plotted against dose and the curves were fitted to a linear-quadratic relationship by a least square analysis.

Results and Discussion

Total dsb in both cell lines, HMV-I and HA-1, increased with dose of ^{60}Co γ-rays(Fig. 1). No. dsb was, however, independent on radiation sensitivity of cell killing shown in Fig. 2, because dsb induced much more in HA-1 cells than in HMV-I cells which were more radiosensitive in cell killing than HA-1 cells. About

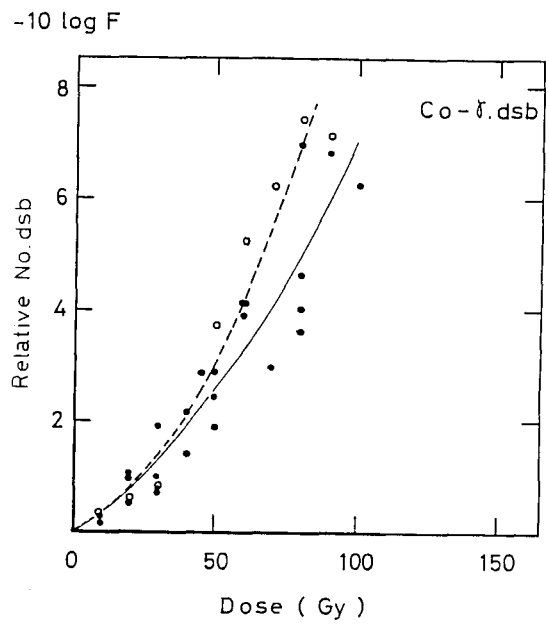

Fig. 1 Relative number of total DNA double strand breaks induced by ^{60}Co γ-rays. ○, HA-1; ●, HMV-I.

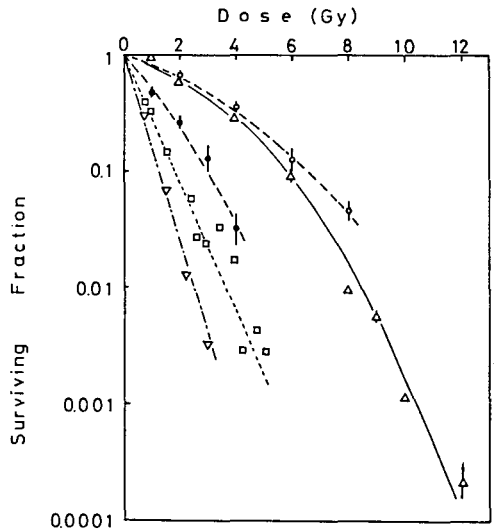

Fig. 2 Dose-survival curves. ○, HA-1 irradiated with ^{60}Co γ-rays; ●, HA-1 irradiated with α-particles; △, HMV-I irradiated with ^{60}Co γ-rays; ▽, HMV-I irradiated with α-particles; □, HMV-I irradiated with N-ions.

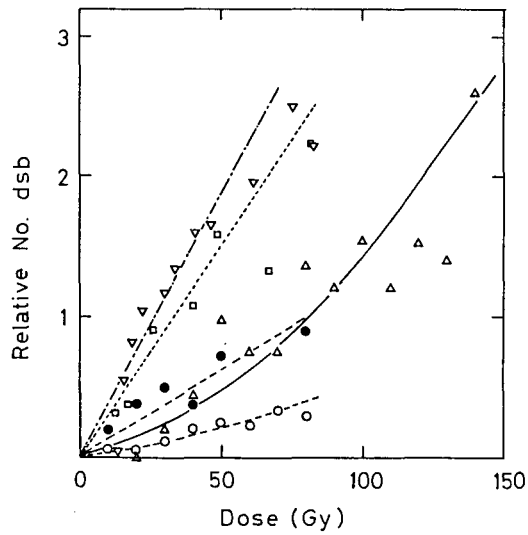

Fig. 3 Relative number of non-repairable DNA double strand breaks. Symbols are same as Fig. 2.

60-90% of total dsb was rejoining during post-irradiation incubation at 37°C. Remainder can be considered to be non-repairable. It was shown that the non-repairable dsb in both cells increased quadratically with dose of γ-rays, whereas increased linearly with dose of charged particle radiation (Fig. 3). Five curves shown in Fig. 3 were in order of radiosensitivity of cell killing and were mirror images of dose-survival curves. We calculated the number of non-repairable dsb at D_{37} in two cell lines which had different radiosensitivity. The D_{37} doses varied with the cell line and kind of radiations from 0.5 Gy (α-particles with LET of 77 keV/μm, HMV-I) to 4 Gy (γ-rays, HA-1). But the number of non-repairable dsb per cell per D_{37} was almost constant instead of difference in cell lines and kind of radiation. These results shows the close relationship between the cell survival and non-repairable dsb.

1. Ritter et al., Nature 266, 653-655, 1977.
2. Roots et al., Radiat. Res. 78, 38-49, 1979.
3. Eguchi et al. Int. J. Radiat. Biol. in press.
4. Ohno et al., Int. J. Radiat. Biol. 45, 21-26, 1984.
5. Yatagai et al., Radiat. Res. 77, 250-258, 1979.
6. Bladely and Kohn, Nucl. Acid Res. 7, 793-804, 1979.