

# Kinetics of chromatid break repair in G2-human fibroblasts exposed to low- and high-LET radiations

T. Kawata<sup>1</sup>, M. Durante<sup>2</sup>, K. George<sup>1,3</sup>, Y. Furusawa<sup>4</sup>, E. Gotoh<sup>5</sup>, N. Takai<sup>4</sup>, H. Wu<sup>1,6</sup>, F.A. Cucinotta<sup>1</sup>

1. NASA Lyndon B. Johnson Space Center, Radiation Biophysics Laboratory (USA)

2. Department of Physics, University "Federico II", Naples (Italy)

3. Wyle laboratories, Houston, TX (USA)

4. International Space Radiation Laboratory, National Institute of Radiological Sciences, Chiba (Japan)

5. Division of Genetic Resources, National Institute of Infectious Diseases, Tokyo (Japan)

6. Kelsey-Seybold Clinic, Houston, TX (USA)

## Abstract

The purpose of this study is to determine the kinetics of chromatid break rejoining following exposure to radiations of different quality. Exponentially growing human fibroblast cells AG1522 were irradiated with  $\gamma$ -rays, energetic carbon (290 MeV/u), silicon (490 MeV/u) and iron (200 MeV/u, 600 MeV/u). Chromosomes were prematurely condensed using calyculin A. Prematurely condensed chromosomes were collected after several post-irradiation incubation times, ranging from 5 to 600 minutes, and the number of chromatid breaks and exchanges in G2 cells were scored. The relative biological effectiveness (RBE) for initial chromatid breaks per unit dose showed LET dependency having a peak at 55 keV/ $\mu$ m silicon (2.4) or 80 keV/ $\mu$ m carbon particles (2.4) and then decreased with increasing LET. The kinetics of chromatid break rejoining following low- or high-LET irradiation consisted of two exponential components. Chromatid breaks decreased rapidly after exposure, and then continued to decrease at a slower rate. The rejoining kinetics was similar for exposure to each type of radiation, although the rate of unrejoined breaks was higher for high-LET radiation. Chromatid exchanges were also formed quickly.

KEYWORDS: Premature chromosome condensation, High-LET radiations, Chromatid breaks and exchanges.

## 1. Introduction

Recently, a new PCC technique has been developed to analyze chromosome aberrations induced by radiation [1], in which chromosome condensation is induced by Calyculin-A. Calyculin A is capable of inducing PCC in many types of cell [2] [3] [4]. This chemical-induced PCC technique is technically much easier than cell-fusion PCC technique, induces a higher PCC index [1] and can induce PCC in different phases of the cell cycle [2] [3]. In the present study, we apply this technique to study the initial G2-chromosome aberrations and repair kinetics following exposure to radiations of different LET.

## 2. Material and methods

Exponentially growing AG1522 cells were irradiated with  $\gamma$ -rays (10Gy/min) or heavy ions (around 1Gy/min). Irradiation with heavy ions was performed at Heavy Ion Medical Accelerator in Chiba (HIMAC) and at Brookhaven National Laboratory. The beam energy was 290 MeV/u for carbon, 490 MeV/u (55 keV/ $\mu$ m) for silicon, 200 MeV/u (440 keV/ $\mu$ m) and 600 MeV/u (185 keV/ $\mu$ m) for iron, respectively. Carbon particles were accelerated to 290 MeV/u and shielded with 147 mm water-equivalent absorber to obtain a higher LET value (80 keV/ $\mu$ m). The doses used were 2Gy for  $\gamma$ -rays, 1Gy for silicon, 1.5Gy for each iron and 2Gy for carbon

particles. After irradiation, cells were allowed for repair for various times and then chromosomes were forced to condense prematurely using calyculin A as described previously [5]. Briefly, 50 nM calyculin A was added to the growth medium and cells were incubated for 30 minutes at 37 °C. Cells were then swollen in 75 mM KCl for 20 minutes at 37 °C, and fixed with methanol: glacial acetic acid (3:1 vol./vol.). Cells were dropped on a clean slide and stained with Giemsa. Chromatid-type breaks and isochromatid-type breaks were scored separately. One isochromatid break was scored to be two chromatid breaks.

## 3. Results

In figure 1, the number of initial chromatid-type breaks and isochromatid breaks per unit dose following exposure to different type of radiations is shown. The peak for chromatid break induction was observed at 55 keV/ $\mu$ m silicon and 80 keV/ $\mu$ m carbon particles. The relative biological effectiveness (RBE) ranged from 1.5 (440 keV/ $\mu$ m iron) to 2.4 (55 keV/ $\mu$ m silicon and 80 keV/ $\mu$ m iron). Compared with low-LET radiation, the fraction of the isochromatid breaks out of total breaks was found to be much higher for high-LET radiations. Figure 2 shows the repair kinetics of chromatid breaks following exposure to radiations of different LET. Although the percentage of residual breaks in high-LET irradiated cells was apparently higher

**Table I** – Fitting parameters and time constants of repair kinetics.

Radiation	LET keV/ $\mu\text{m}$	Dose (Gy)	A	B	C	$\tau_1$ (min)	$\tau_2$ (min)	$\chi^2$	Degrees of freedom
$\gamma$ -rays	0.6	2	49.9 $\pm$ 3.4	47.3 $\pm$ 3.4	2.9 $\pm$ 3.7	6.0 $\pm$ 0.9	227 $\pm$ 48	42.5	7
Silicon	55	1	30.1 $\pm$ 3.2	57.5 $\pm$ 2.9	12.5 $\pm$ 3.1	6.6 $\pm$ 1.8	206 $\pm$ 37	6.7	2
Carbon	80	2	26.6 $\pm$ 5.4	61.6 $\pm$ 5.1	12.2 $\pm$ 4.6	4.8 $\pm$ 2.2	166 $\pm$ 46	51.6	4
Iron	440	1.5	24.1 $\pm$ 6.0	58.3 $\pm$ 7.4	18.0 $\pm$ 8.7	4.7 $\pm$ 2.6	208 $\pm$ 82	66.3	4

$\tau_1$ : Time constant of fast component

$\tau_2$ : Time constant of slow component

Fitting function:  $y = A \exp(-t/\tau_1) + B \exp(-t/\tau_2) + C$ .

Half time is calculated as  $0.692 * \tau$  and shown.

Errors correspond to  $\chi^2$  variations of unity.

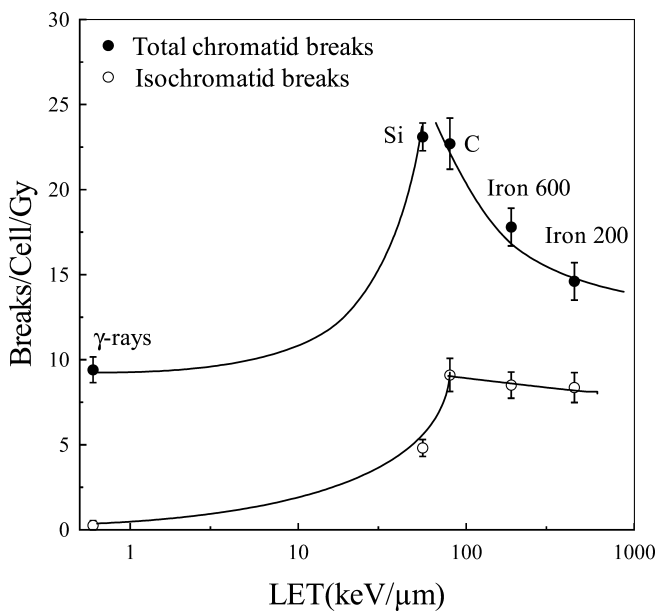


Fig. 1 – Initial yields of total chromatid breaks and isochromatid breaks after exposure to radiations with different LET. Bars are standard errors of the mean.

compared to  $\gamma$ -rays, there was no statistically significant difference in the fast and slow time constant between low- and high-LET radiations ( $p > 0.01$ ). The fitted parameters and time constants are shown in the table. In figure 3, the kinetics of chromatid-type exchange formation is shown. Chromatid exchange formation was formed quickly, and for 440 keV/ $\mu\text{m}$  iron, a peak was observed at 180 minutes after irradiation.

#### 4. Discussion and Conclusion

Using a new chemical-induced PCC technique, we could measure the number of chromatid breaks induced by low- and high-LET radiations and could determine the repair kinetics after different incubation times. Although kinetics after high-LET exposure showed the similar fast and slow time constants

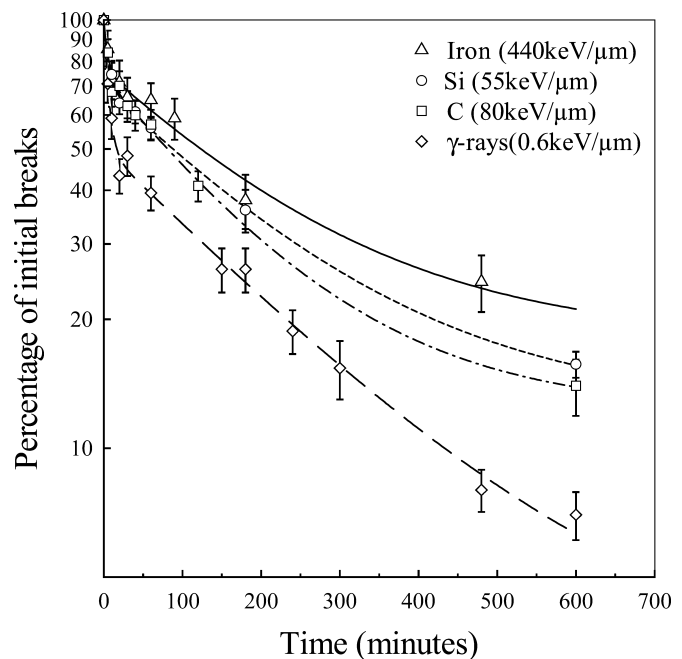


Fig. 2 – Kinetics of rejoining of chromatid breaks after irradiation as a function of incubation time. Bars are standard errors of the mean.

seen with  $\gamma$ -rays, the percentage of residual breaks was from 4 to 6 times higher. The tendency of higher rate of residual breaks for high-LET radiations is in good agreement with the results of Goodwin and colleagues [6]. They reported that the levels of residual fragments in G1 Chinese hamster cells were LET dependent. The reported percentage of residual excess fragments was 49% after exposure to 183 keV/ $\mu\text{m}$  neon particles and 11% after X-rays exposure. We have also shown that the formation of chromatid-type exchanges occurs very quickly after exposure to various types of radiation. The kinetics of chromatid exchange formation seems to be well correlated with the rapid component of repair kinetics for chromatid breaks.

It has been also found that high-LET radiations are more effective at producing total chromatid breaks, especially isochromatid breaks. The incre-

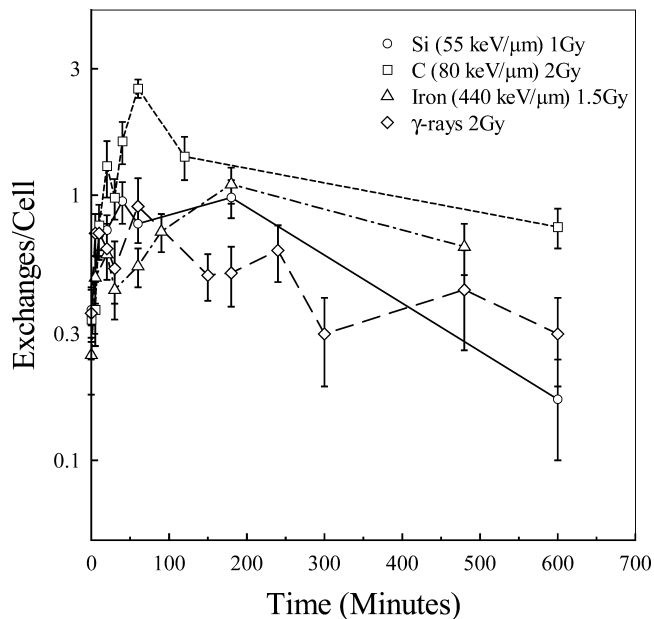


Fig. 3 – Kinetics of formation of chromatid-type exchanges after exposure to each type of radiation. Bars are standard errors of the mean.

ased production of isochromatid breaks has been also reported by Durante and colleagues [7] and Griffin and colleagues [8]. Using metaphase technique, both groups have found that isochromatid deletions were more frequently produced by alpha particles than X-rays. An increased production of isochromatid breaks by high-LET radiation could be attributed to the different patterns of energy depositions for sparsely and densely ionizing radiations. It could be concluded that an effective production of isochromatid break would be a signature of high-LET radiation exposure.

### Acknowledgments

This work was supported by NASA Space Radiation Health Program. T.K. is supported by an NRC grant (fellowship n. 9818170).

### REFERENCE

- [1] Durante M, Furusawa Y, Gotoh E. A simple method for simultaneous interphase-metaphase chromosome analysis in biodosimetry. *Int J Radiat Biol* 1998; 74; 457-462.
- [2] Gotoh E, Asakawa Y, Kosaka H. Inhibition of protein serine/threonine phosphatases directly induces premature chromosome condensation in mammalian somatic cells. *Biomed Res* 1995; 16; 63-68.
- [3] Coco-Martin JM, Begg AC. Detection of radiation-induced chromosome aberrations using fluorescence *in situ* hybridization in drug-induced premature chromosome condensations of tumour cell lines with different radiosensitivities. *Int J Radiat Biol* 1997; 71; 265-273.
- [4] Alsbeih G, Raaphorst GP. Differential induction of premature chromosome condensation by calyculin A in human fibroblast and tumor cell lines. *Anticancer Res* 1999; 19; 903-908.
- [5] Gotoh E, Kawata T, Durante M. Chromatid break rejoining and exchange aberration formation following  $\gamma$ -ray exposure: analysis in G2 human fibroblasts by chemical-induced premature chromosome condensation. *Int J Radiat Biol* 1999; 75; 1129-1135.
- [6] Goodwin EH, Blakely EA, Tobias CA. Chromosomal damage and repair in G1-phase Chinese hamster ovary cells exposed to charged-particle beams. *Radiat Res* 1994; 138; 343-351.
- [7] Durante M, Gialanella G, Grossi GF, Nappo M, Pugliese M, Bettega D, Calzolari P, Chiorda GN, Ottolenghi A, Tallone-Lombardi L. 1994, Radiation-induced chromosomal aberrations in mouse 10T1/2 cells: dependence on the cell-cycle stage at the time of irradiation. *Int J Radiat Biol* 1994; 65; 437-447.
- [8] Griffin CS, Harvey AN, Savage JRK. 1994, Chromatid damage induced by  $^{238}\text{Pu}$   $\alpha$ -particles in G2 and S phase Chinese hamster V79 cells. *Int J Radiat Biol* 1994; 66; 85-98.