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## **Radiation-Induced Chromosome Damage in G<sub>1</sub> Phase Cells as Breaks in Premature Chromosome Condensation (PCC) and Its Biological Meaning**

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### **Premature chromosome condensation (PCC)/chromatid aberration/high LET radiations/mutation/morphological transformation**

We studied radiation-induced chromosome fragmentation in the G<sub>1</sub>-phase of Syrian golden hamster embryo (SHE) cells by premature chromosome condensation (PCC). SHE cells were irradiated with <sup>14</sup>N ions (95 MeV) and <sup>4</sup>He ions (22 MeV) generated by the cyclotron and <sup>137</sup>Cs gamma rays. The number of chromosome breaks in PCC (PCC break) induced by <sup>14</sup>N ions was higher than in those by the others. High linear energy transfer (LET) radiation (heavy ions) is more effective in the induction of PCC breaks than low LET radiation (gamma-rays). 530 keV/μm <sup>14</sup>N induced PCC 2.4 times more than <sup>137</sup>Cs gamma rays. There is an inverse relationship between the number of chromatid delations and the incidence of mutation or transformation. There is, however, a good correlation between the incidence of PCC breaks and frequencies of mutation and/or morphological transformation. Although over 95% of the PCC breaks induced by gamma-rays were rejoined within 8 hours of post-irradiation incubation, only 35 to 45% of PCC breaks induced by heavy ions were rejoined. These results suggest that there is a qualitative difference in the initial chromosome damage caused by heavy ions and gamma rays.

### **INTRODUCTION**

It is well known that chromosomal translocations, numerical anomalies and deletions have implications for rearrangements, duplications or deletions of specific genes, including oncogenes, genes for growth factor proteins and cancer development. The precise mechanism of cancer induction is not yet defined, but there is powerful evidence to suggest that chromosomal damage is an important initial step<sup>1-4</sup>). Therefore, the main objective of a chromosome aberration assay is to make a toxicological database for genotoxicity which can be used for human safety evaluation.

However, there are significant problems in using chromosome analysis which must be

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recognized. A major problem involves the position of the cell cycle, which would affect the incidence of chromosome aberrations. Because chromosomes condense only at the mitotic phase of the cell cycle, chromosome damage in interphase can not be directly measured by the conventional chromosome analysis. Therefore, if cells are exposed to a specific radiation which causes severe cell cycle delay and/or interphase death, the frequency of chromosomal damage would be underestimated.

Recently, X-ray-induced damage has been investigated using PCC<sup>5-7)</sup> and the data suggested that it is a very sensitive method to detect chromosome damage in interphase cells. Therefore, in this study, we detected chromosome aberrations as chromatin breaks in interphase cells using PCC and compared it with the data of those detected by conventional chromosome analysis. We discuss the usefulness of PCC in measuring genotoxicity.

### MATERIALS AND METHODS

*Cells:* Primary Syrian golden hamster embryo (SHE) cells obtained by trypsinization of 13- to 14-day old embryo<sup>8)</sup> were used as target cells. Cells were cultured in Eagle's minimum essential medium supplemented with 10% fetal bovine serum in a 5% CO<sub>2</sub> incubator at 37°C.

*Irradiation:*  $6 \times 10^5$  SHE cells were inoculated into a 35-mm plastic dish. Two days later, cells reached a density of about  $4 \times 10^5$  per cm<sup>2</sup> and were used to prepare cultures for experiments. Flow cytometry measurements indicated that plateau-phase cultures of SHE cells contained about 90% cells in G<sub>1</sub> phase and 10% in other phases. The 95 MeV <sup>14</sup>N ions and 22 MeV <sup>4</sup>He ions were generated by the 160 cm cyclotron at the Institute of Physical and Chemical Research in Japan. Dosimetry and irradiation procedures were performed according to methods described elsewhere<sup>9-11)</sup>. Briefly, we measured the particle fluency by solid state detectors, and the beam energy and LET value at the sample position were calculated using the range-energy tables given by Northcliffe and Schilling<sup>12)</sup>, taking into consideration the density of the air and water above the cells. We also monitored the beam currents by transmission ionization chambers and converted them into absorbed dose. We estimated the beam energy and LET value at the sample position to be 3.1 MeV/n and 530 keV/ $\mu$ m for <sup>4</sup>N ions, 4.2 MeV/n and 36 keV/ $\mu$ m for <sup>4</sup>He ions without the 100  $\mu$ m Al absorber, and 1.7 MeV/n and 77 keV/ $\mu$ m for <sup>4</sup>He ions with the absorber. The dose rates of <sup>14</sup>N and <sup>4</sup>He ions irradiation were 0.3 Gy/min and 1.0 Gy/min, respectively. SHE cells were irradiated with <sup>137</sup>Cs gamma rays at a dose rate of 1.4 Gy/min. All irradiation were carried out at room temperature.

*Induction of premature chromosome condensation (PCC):* The procedure for polyethylene glycol (PEG) mediated cell fusion and induction of PCC have been described in detail elsewhere<sup>13)</sup>. Briefly, mitotic CHO cells harvested from culture treated with 0.1  $\mu$ g/ml of colcemid (Demecolcin; Wako pure chemical industries, Ltd., Osaka) for 3 hours, were mixed with an equal number of irradiated SHE cells in a polypropylene tube (Falcon 2059). The cell mixture was centrifuged at 1,000 rpm for 5 min and the pellet was washed in 4 ml PBS. After

centrifugation at 1,000 rpm for 5 min, the pellet was exposed for 1 min to 0.15 ml of PEG in 75 mM Hepes 50% w/v (MW=1,540, Boehringer Mannheim GmbH, W. Germany). Four milliliters of PBS was gently added, and the cell suspension was centrifuged at 1,000 rpm for 5 min. The pellet was resuspended in 4 ml of MEM containing 0.1  $\mu\text{g}/\text{ml}$  of demecolcin and incubated at 37°C for 1hr. Subsequently, cells were treated with 0.075 M KCl solution for 20 min at room temperature and fixed in Carnoy's solution. The cell solution was dropped onto slides, air dried, and stained with 5% Giemsa. PCC samples were scored under a light microscope.

*Chromosome analysis:* Chromosome samples were prepared by a standard air-drying method<sup>2)</sup>. After post-irradiation incubation, cells were treated by a final concentration of 0.2  $\mu\text{g}/\text{ml}$  of colcemid and incubated for 2 hours. The number of chromatid aberrations (gaps, delations and translocations) were scored in at least 100 metaphase per sample.

*Transformation Assay:* The transformation assay used in this experiments was as reported previously<sup>2,8)</sup>. After irradiation, cells from some of dishes inoculated into 90-mm plastic dishes, containing  $10^4$  feeder cells at cloning density (100–3,000 viable cells) to determine both the frequency of morphological transformation and the lethal effects. The frequency of transformants was expressed as the ratio of the number of morphologically transformed type B colonies to the total number of colonies counted as previously described<sup>8)</sup>.

*Mutation Assay:* The mutation assay used in the experiments was as reported previously<sup>14,15)</sup>. When cells had divided at least three times after irradiation,  $10^6$  cells were replated into 40 plates (100-mm diameter) with 10 ml of selective medium containing 40 mM of 6-thioguanine (6TG) and cultured for a further 20 days to determine mutation frequency. The frequency of mutants was expressed as the ratio of the number of mutants to  $10^6$  survivors.

## RESULTS AND DISCUSSION

The precise mechanism of cancer induction is not yet defined, but there is powerful evidence to suggest that chromosomal damage is an important initial step<sup>1–4)</sup>. Therefore, the main objective of a chromosome aberration assay is to make a toxicological database for mutagenic and/or carcinogenic toxicity which can be used for human safety evaluation.

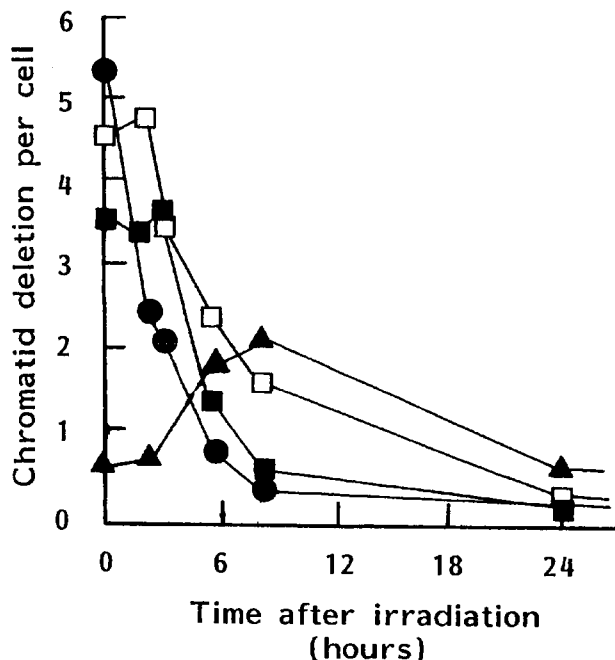
We have found that high LET heavy ions are more effective in inducing mutation<sup>19)</sup> and neoplastic transformation<sup>9)</sup> *in vitro* as reported previously<sup>16–18)</sup>. Therefore, we predicted that much more chromosomal damage would be induced by high LET, as reported previously<sup>20,21)</sup>. However, the induction of all types of chromatid aberration (gap, deletion and exchange) by high LET radiations was lower than that by  $^{137}\text{Cs}$  gamma-rays<sup>10,22)</sup>. The RBE for high LET radiation for chromatid deletions to low LET radiation were 0.64 for 36 keV/ $\mu\text{m}$   $^4\text{He}$ , 0.83 for 77 keV/ $\mu\text{m}$   $^4\text{He}$  and 0.24 for 530 keV/ $\mu\text{m}$   $^{14}\text{N}^{22)}$  on the basis of a same absorbed dose (2 Gy). There is an inverse relationship between the incidence of mutation or transformation and the

**Table I.** Comparison of the frequency of chromosome aberrations and mutants and/or transformants induced in SHE cells by 2 Gy of various LET radiations.

Radiation	Lethal fraction	Mutants <sup>a)</sup>	Transformants <sup>b)</sup>	Chromatid breaks <sup>c)</sup>	PCC breaks <sup>d)</sup>
Gamma ( <sup>60</sup> Co)	0.40(1.3) <sup>e)</sup>	96(1.1)	1,750(1.0)	6.5(1.2)	—
Gamma( <sup>137</sup> Cs)	0.32(1.0)	87(1.0)	1,700(1.0)	5.4(1.0)	13.8(1.0)
<sup>4</sup> He(36 keV/ $\mu$ m)	0.83(2.6)	424(4.9)	3,900(2.3)	3.5(.64)	18.5(1.3)
<sup>4</sup> He(77 keV/ $\mu$ m)	0.86(2.7)	500(5.8)	4,300(2.5)	4.5(.83)	27.0(2.0)
<sup>14</sup> N(530 keV/ $\mu$ m)	0.81(2.5)	650(7.5)	5,400(3.2)	1.3(.24)	29.4(2.1)

a) and b), per 10<sup>6</sup> survivors, c) and d), per cell. e) Relative biological effectiveness (RBE) to <sup>137</sup>Cs.

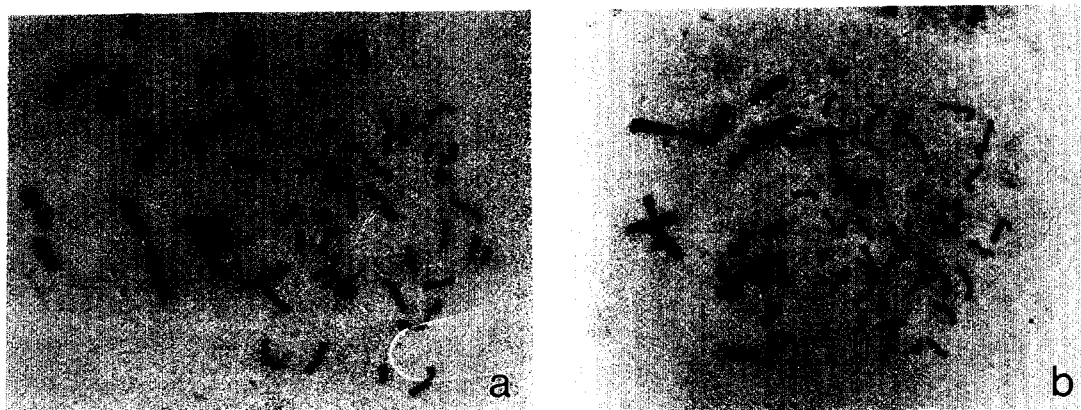
number of chromatid deletions in SHE cells irradiated with a dose of 2 Gy (Table I). Because chromosomes condense only at the mitotic phase of the cell cycle, conventional chromosome analysis can not measure chromosome damage in interphase. Therefore, if cells are exposed to a specific radiation which causes severe cell cycle delay and/or interphase death as reported<sup>23,24)</sup>, the frequency of chromosomal damage will be underestimated. We examined the incidence of chromosome aberration (chromatid and chromosome aberrations) in SHE cells irradiated with 2 Gy of various LET radiations during post-irradiation incubation, and results were shown in Figure 1. Cells were treated with colcemid to accumulate mitotic cells for 2 hours just before



**Fig. 1.** The effect of changing the length of post-irradiation incubation prior to chromosome assay on the incidence of chromosome aberrations in SHE cells irradiated with 2 Gy of various LET radiations. Cells were treated with colcemid for 2 hours just before chromosome sampling. Symbols: <sup>137</sup>Cs gamma rays (●), 36 keV/ $\mu$ m <sup>4</sup>He ions (■), 77 keV/ $\mu$ m <sup>4</sup>He ions (□), and 530 keV/ $\mu$ m N ions (▲).

each sampling point. The induction of chromosome aberrations induced by  $^{137}\text{Cs}$  gamma rays and  $^4\text{He}$  were a maximum at 2–4 hours after irradiation, while it by  $^{14}\text{N}$  increased gradually up to 8 hours after irradiation and then gradually decreased with length of cultivation. These results suggest clearly that irradiation with  $^{14}\text{N}$  ions causes severe cell cycle delay than gamma rays and that it is difficult to compare the incidence of chromosome aberrations among different LET radiation.

Therefore, we introduced PCC to directly measure initial chromosome damage in interphase cells. Non-irradiated SHE cells have 44  $G_1$ -PCC fragments as shown in Figure 2a. In irradiated cells, the number of PCC fragments clearly increase (Figure 2b). The yield of induced PCC breaks per cell is estimated as the excess PCC fragments in irradiated  $G_1$  phase cells and results are shown in Table II. The incidence of PCC breaks increase linearly with increasing doses up to 4 Gy. High LET radiation is more effective in inducing PCC breaks than gamma rays. For the example, the number of PCC breaks per cell irradiated with 2 Gy of  $^{14}\text{N}$  (530 keV/ $\mu\text{m}$ ) was approximately 29; whereas that per cells irradiated with 2 Gy of  $^{137}\text{Cs}$  gamma rays was approximately 14. The frequency of 6TC resistant cells induced in SHE cells irradiated with 2 Gy of  $^{14}\text{N}$  was approximately 650/ $10^6$  survivors, but only about 87/ $10^6$  survivors in SHE cells irradiated with 2 Gy of  $^{137}\text{Cs}$  gamma rays<sup>19</sup>). Similarly, for transformation induction, the frequency of morphological transformants induced in SHE cells irradiated with 2



**Fig. 2.** Prematurely condensed chromosomes in normal SHE cells (a) (44 PCC fragments) and irradiated SHE cells with 2 Gy of He ions (36 keV/ $\mu\text{m}$ ) (b) (63 PCC fragments). Normal SHE cells have 44 metaphase chromosomes.

**Table II.** The Induction of PCC breaks in SHE cells irradiated with various LET radiations.

Radiation	Number of PCC breaks in SHE cells irradiated with					
	0	1	2	3	4	(Gy)
gamma( $^{137}\text{Cs}$ )	1.2	12.2	15.2	18.6	23.5	
$^4\text{He}$ (36 keV/ $\mu\text{m}$ )		12.5	20.5	26.5	33.2	
$^4\text{He}$ (77 keV/ $\mu\text{m}$ )		14.1	25.1	36.9	44.6	
$^{14}\text{N}$ (530 keV/ $\mu\text{m}$ )		9.9	30.2	42.2	ND	

Gy of  $^{14}\text{N}$  was approximately  $5,400/10^6$  survivors, but only about  $1,700/10^6$  survivors in SHE cells irradiated with 2 Gy of  $^{137}\text{Cs}$  gamma rays (Table I).

There is a good correlation between the incidence of PCC breaks and the frequency of mutation and/or morphological transformation in SHE cells irradiated with a dose of 2 Gy (Table I). The RBE cells production of mutations and/or transformations was larger than that for the induction of PCC breaks. However, if the incidence of PCC breaks is compared among the differing LET radiations induced by doses adjusted to result in equal cytotoxicity, there is no difference between high and low LET radiation. For example, at a 50% survived dose of  $^{14}\text{N}$ , the frequency of PCC breaks by was about 12/cell; whereas at a 50% survival dose of  $^{137}\text{Cs}$  gamma rays it was about 13/cell.

We examined the repair of PCC breaks during post-irradiation incubation. PCC breaks induced by  $^{137}\text{Cs}$  gamma rays rejoined more rapidly than those induced by high LET radiation (Table III). Over 90% of PCC breaks induced by gamma-rays rejoined within 8 hours of post-irradiation incubation, but in the case of  $^{14}\text{N}$  and  $^4\text{He}$  ions, only 35 to 45% of PCC breaks rejoined 8 hours after irradiation. These results show that the PCC breaks produced by high LET radiation are qualitatively different, so they are less reparable as reported<sup>21)</sup>, or are repaired more slowly than those produced by low LET radiations. Therefore, cells containing this type of breaks would experience severe cell cycle delay or interphase death and should be frequently eliminated from the cell population during progression to the first mitosis after exposure. The reduced incidence of chromatid delations by high LET radiation is probably caused by this reason. In this case, the high LET radiation breaks may remain open for a longer time and have more opportunity to interact. The prolongation of cell cycle allows cells additional time for the expression of lesions that would otherwise remain unexpressed, and could perhaps be associated with the development of additional karyotypic changes and aneuploidy. This process might be higher in cells irradiated by higher LET radiations than in cells irradiated by low LET radiations.

**Table III.** Repair kinetics of PCC breaks in SHE cells irradiated with 2 Gy of various LET radiations.

Radiation	Number of induced PCC breaks at various incubation periods (hour) after exposure to 2 Gy of various LET radiations			
	0	2	4	8
gamma( $^{137}\text{Cs}$ )	13.8(1.0)	5.5(0.4)	1.2(.09)	0.7(.05)
$^4\text{He}$ (36 keV/ $\mu\text{m}$ )	18.5(1.0)	12.0(.64)	11.1(.60)	10.2(.55)
$^4\text{He}$ (77 keV/ $\mu\text{m}$ )	27.0(1.0)	20.8(.77)	20.6(.76)	18.0(.67)
$^{14}\text{N}$ (530 keV/ $\mu\text{m}$ )	29.4(1.0)	23.5(.80)	22.1(.76)	20.2(.69)

a) Relative incidence against initial PCC breaks.

At present, the nature of the lesion resulting in what we observed as a "PCC break" is unknown. It seems likely however, that the RBE of 1.3–2.1 for high LET radiation in the production of initial PCC breaks may be a substantial component of the large RBE for the production of mutations and/or transformations. The remaining, even larger component, is

likely to be due to special or qualitative differences in the breaks themselves. It still remains to be determined what could be the mechanism leading to much mutation and transformation of cells irradiated with high LET radiations. However, PCC is more sensitive in detecting initial chromosome damage induced by radiation than the conventional chromosome analysis and it is very useful in assessing genotoxic risk by radiation and probably also by chemicals.

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### REFERENCES

1. KODAMA, S. and SASAKI, M. S. (1987) The involvement of chromosome 13 in the X-ray-induced *in vitro* transformation of mouse m5S cells. *Jpn. J. Cancer Res.*, **78**: 372–381.
2. WATANABE, M., SUZUKI, K. and KODAMA, S. (1990) Karyotypic changes with neoplastic conversion in morphologically transformed golden hamster embryo cells induced by X-rays. *Cancer Res.* **50**: 760–765.
3. SUZUKI, K., SUZUKI, F., WATKANABE, M. and NIKAIDO, O. (1989) Multistep nature of X-ray induced neoplastic transformation in golden hamster embryo cells: expression of transformed phenotypes and stepwise changes in karyotypes. *Cancer Res.*, **49**: 2134–2140.
4. SUZUKI, K., YASUDA, N., SUZUKI, F., NIKAIDO, O. and WATANABE, M. (1989) Trisomy of chromosome 9q: specific chromosome change associated with tumorigenicity during the process of X-ray-induced neoplastic transformation in golden hamster embryo cells. *Int. J. Cancer*, **44**: 1057–1061.
5. WALDREN, C. A. and JOHNSON, R. T. (1974) Analysis of interphase chromosome damage by means of premature chromosome condensation after X- and ultraviolet-irradiation. *Proc. Nat. Acad. Sci. USA*, **71**: 1137–1141.
6. HITTELMAN, W. N. and RAO, P. N. (1974) Premature chromosome condensation. I. visualization of X-ray-induced chromosome damage in interphase cells. *Mutation Res.* **23**: 251–258.
7. PANTELIAS, G. E. and MAILLIE, H. D. (1985) Direct analysis of radiation-induced chromosome fragments and rings in unstimulated human peripheral blood lymphocytes by means of the premature chromosome condensation technique. *Mutation Res.* **140**: 67–72.
8. WATANABE, M., HORIKAWA, M. and NIKAIDO, O. (1984) Induction of oncogenic transformation by low doses of X rays and dose-rate effect. *Radiation Res.* **98**: 274–283.
9. SUZUKI, M., WATANABE, M., SUZUKI, K., NAKANO, K. and KANEKO, I. (1989) Neoplastic cell transformation by heavy ions. *Radiation Res.* **120**: 468–476.
10. SUZUKI, K., SUZUKI, M., NAKANO, K., KANEKO, I. and WATANABE, M. (1990) Analysis of chromatid damages in G<sub>2</sub> phase induced by heavy ions and X-rays. *Int. J. Radiat. Biol.* **58**: 781–789.
11. EGUCHI, K., INADA, T., YAGUCHI, Y., SATOH, S. and KANEKO, I. (1987) Induction and repair of DNA lesions in cultured human melanoma cells exposed to nitrogen-ion beams. *Int. J. Radiat. Biol.* **52**: 115–123.
12. NORTHCLIFFE, L. C. and SCHILLING, R. F. (1970) Range and stopping power tables for heavy ions. *Nuclear Data Tables*, A7, 233–463.

13. PANTELIAS, G. E. and MAILLIE, H. D. (1983) A simple method for premature chromosome condensation induction in primary human and rodent cells using polyethylene glycol. *Somatic Cell Gene.* **9**: 533-547.
14. WATANABE, M., MAHER, V. M. and MCCORMICK, J. J. (1985) Excision repair of UV- or benzo[a]pyrene-diol epoxide-induced lesions in xeroderma pigmentosum variant cells is "error free". *Mutation Res.* **146**: 285-294.
15. WATANABE, M. and SUZUKI, K. (1991) Expression dynamics of transforming phenotypes in X-irradiated Syrian hamster embryo cells. *Mutation Res.*, **249**: 71-80.
16. SKARSGARD, L. D., KIHLMAN, B. A., PARKER, L., PUJARA, C. M. and RICHARDSON, S. (1967) Survival, chromosome abnormalities, and recovery in heavy-ion and X-irradiated mammalian cells. *Radiation Res. Suppl.* **7**: 208-221.
17. RITTER, M. A., CLEAVER, J. E. and TOBIAS, C. A. (1977) High-LET radiations induce a large proportion of nonrejoining DNA breaks. *Nature*, **266**: 653-655.
18. ROOTS, R., YANG, T. C., GRAISE, L., BLAKLY, E. A. and TOBIAS, C. A. (1979) Impaired Repair capacity of DNA breaks induced in mammalian cellular DNA by accelerated heavy ions. *Radiation Res.* **78**: 38-49.
19. WATANABE, M., SUZUKI, K., SUZUKI, M., KOSAKA, T., NAKANO, K. and KANEKO, I. (1991) *In vitro* cell transformation and mutation by heavy ions. *RIKEN Accel. Prog. Rep.*, **22**: 141-143.
20. KRAFT, G. (1987) Radiobiological effects of heavy ions: Inactivation, induction of chromosome aberrations and strand breaks. *Nuclear Science Applications*, **3**: 1-28.
21. GOODWIN, E., BLAKELY, E., IVERY, J. and COX, R. (1989) Repair and misrepair of heavy ion-induced chromosomal damage. *Advances in Space Research*, **9**: 83-89.
22. WATANABE, M., SUZUKI, M., SUZUKI, K. and WATANABE, K. (1991) Comparison of the efficiency of mutants and/or transformants and chromosome damage induced in SHE cells by various LET radiations, *In Vitro Toxicol.*, **4**: 93-100.
23. GOODHEAD, D. T., THAKER, J. and COX, R. (1978) Non rejoining DNA breaks and cell inactivation. *Nature (London)*, **272**: 379-380.
24. ROOTS, R., YANG, T. C., CRAISE, L., BLAKELY, E. A. and TOBIAS, C. (1979) Impaired repair capacity of DNA breaks induced in mammalian cellular DNA by accelerated heavy ions. *Radiation Res.* **78**: 38-49.