Induction of Chromosomal Aberrations in the Somatic and Germ Cells of Mice after Long-Term Exposure to Low-Dose-Rate Gamma-Irradiation

HALA F. ABDEL HAMID [a], ADEL ASHOUR[b], HASSAN MOAWAD[c] AND ABDELMOHSEN M. SOLIMAN[d*]

[a] Chemistry of Pesticides Dept. [b] Clinical Genetics dept. [c]Agricultural Biotechnology Dept. [d] Therapeutic Chemistry Dept. National Research Centre, El-Behoos St., Dokki, Cairo, Egypt. P.O.Box: 12622
[*] solimanmohsen@yahoo.com

Abstract: The induction of chromosomal aberrations in the bone marrow and spleen (as somatic cells) and in the spermatocytes (as germ cells) was used to evaluate the mutagenic effects after long-term exposure to low-dose-rate gamma-irradiation in mice. Chromosome aberration rate in mouse splenocytes after long-term exposure to low-dose-rate (LDR) gamma-rays was serially determined by conventional Giemsa method and the fluorescence *in situ* hybridisation (FISH) technique. Incidence of dicentrics and centric rings increased almost linearly up to 8000 mGy following irradiation for about 100 days at an LDR of 20 mGy/day. It was found that long-term exposure to (LDR) gamma-rays induced a marked changes in the percentage of aberrations in both somatic and germ cells. Clear dose-rate effects were observed in the chromosome aberration frequencies between dose rates of 40 mGy/day and 200 Gy/day. Furthermore, the frequencies of complex aberrations increased as accumulated doses increased in LDR irradiation. Chromosome aberrations seem to be induced indirectly after radiation exposure and thus the results indicate that continuous gamma-ray irradiation for 100 days at LDR of 20 mGy/day induced chromosomal instability in mice. These results are important to evaluate the biological effects of long-term exposure to LDR radiation in humans. [Journal of American Science 2010;6(10):860-866]. (ISSN: 1545-1003).

Keywords: chromosomal aberrations, low dose radiation LDS, FISH, chromosome instability, micronucleus.

1. Introduction:

Chronological changes of chromosomal aberration rates related to accumulated doses of a low-dose-rate (LDR) of radiation have not been well studied in animals or humans (Tanaka *etal* 2008). Chromosome instability has been identified as a genetic instability induced by radiation after several cell divisions in cultured rodent and human cells (Kadhim *etal* 1992 and 1994), which is also expressed as hypoxanthine-guanine phosphoribosyltransferase (HPRT) mutations and apoptosis (Seymore *etal* 1986 and Gorgojo *etal* 1989). It is well known that delayed (or late-arising) chromosome aberrations are induced by acute irradiation at a high dose rate after several cell divisions in cultured human cells (Morgan *etal* 1996) and also in mice(Niwa, 2003). Radiation induced genomic (chromosome) instability is defined as nonclonal DNA (chromosomal) damage that arises or increases in several cell generations after exposure to radiation (Mozdarani *etal* 2009). Genetic (chromosome) instability might be related to cancer, although the mechanism is not understood (Jacquet, 2004). However, little information is available regarding chromosome instability *in vivo* induced by long-term irradiation with γ – ray at LDR, although it will be important for the assessment of low-dose radiation in human health (Forand *etal* 2004).

Cytogenetic methods, such as conventional chromosome analysis (Zhang *etal* 2006), micronucleus assay (Fomenko *etal* 2006) and the fluorescence *in situ* hybridisation (FISH) technique (Barrans etal 2003) have been used as suitable methods for evaluating delayed chromosome aberrations, because they can score the aberration rate at each cell level and measure its distribution among cells under observation. The present study was performed in mice with the use of ^{137}Cs γ – rays irradiations at an LDR of 20 mGy/22h/day (0.91 mGy/h) for several tens to hundreds of days, up to 400 days, to observe chronological changes of chromosome rates related with accumulated dose and to observe whether chromosome instability can be induced *in vivo*.

2. Materials and methods

Mice

Male albino mice 7-week-old weighing 18-20 g were used in the present study. The experiment was done in the National Research Center animal house in wide cages and well aerated rooms and they will be fed on special diets and clean water.

Mice were used for long- or short-term irradiation with ^{137}Cs γ – rays irradiation.

Radiation exposure

At least 5-7 mice were grouped for irradiation with each total dose together with non-irradiated mice as controls. Gamma-irradiation was performed using a 137Cs ┐-ray irradiation device (74GBq and 740GBq, respectively; Yoshizawa LA Co. Ltd., Japan). Groups of mice were irradiated with total doses of 100, 140, 250, 500, 750, 800, 1000, 2000, 4000, 6000, and 8000 mGy at dose rate of 20 mGy/22h/day (0.91 mGy/h) for 1- 40 days with the use of ^{137}Cs γ – ray irradiation device. The daily dose of 200 mGy/day and 20 mGy/day, respectively, is described in this paper.

Spleen cell cultures

Mice were sacrificed under ether anaesthesia immediately after the cessation of irradiation, and spleens were sterilely removed. All reagents were obtained from Sigma, St Louis, MI, USA except where noted. For chromosome analysis, spleen cells were isolated and cultured in RPMI 1640 medium (Invitrogen Corp., Carlsbad, CA, USA) containing lipopolysacharide (LPS, 10 g/ml), concanavalin A (ConA, 3 g/ml), and 2-mercapto-ethanol (2-ME, 50 M) under 5% CO_2 atmosphere with 95% humidity at 37˚ C. For micronucleus assay, cytochalasin B (0.5 g/ml) which is a cell-permeable mycotoxin. that inhibits cytoplasmic division by blocking the formation of contractile microfilaments, was added 24 h before harvesting to obtain binucleated cells.

Spermatocytes chromosomal preparations

Mice were sacrificed under ether anaesthesia 2 hrs after the cessation of irradiation. The testes were removed and meiotic chromosomal preparations were done according to the air dry technique (Evans *etal* 1964).

Conventional Giemsa staining method

The conventional Giemsa staining method (Zhang *etal* 2006) was suitable for the analysis of large numbers of chromosome metaphases of LDR-irradiated mice, because the procedure is simple and rapid. Chromosomal aberrations such as dicentric chromosomes (Dic), centric ring chromosomes (Rc), acentric ring chromosomes (Ra), fragments,minutes (min) and deletions (del), hyperploids and chromatid-type aberrations such as gaps (ctg), breaks (ctb) and exchanges (exc) were observed in 500–1000 metaphases per mouse under a light microscope (Olympus Bx50, Olympus, Tokyo, Japan).

Micronucleus assay in spleen and bone marrow

Three to five mice were analysed in each experimental group at the total doses of 5000, 6000 and 8000 mGy with irradiation at a dose rate of 20 mGy/day by micronucleus assay to observe micronucleus incidence in splenocytes. Non-irradiated mice of the same age were also analysed as a control group.

Interphase fluorescence in situ hybridization (FISH) analysis

Five mice were analysed at 8000 mGy with irradiation of the 20 mGy/day dose rate by FISH method (Barrans *etal* 2003) using centromere probes of chromosomes 5, 15, and 18 to observe the numerical chromosome aberration rate in splenocytes. Five non-irradiated control mice of the same age as irradiated mice were used as a control.

Statistical analysis

The standard error for yields of chromosome aberrations was estimated by regression analysis (Sen and Srivastava, 1990).

3. Results and Discussion:

Incidences of dicentric and centric ring chromosomes

Total aberrations of dicentric and centric rings in mice exposed to γ – rays at the dose rate of either 200 mGy/day or 20 mGy/day and age-matched non-irradiated control mice were estimated. The frequencies of aberrations per 100 cells at 8000 mGy at 20 mGy/day were 17.2 times higher than those of non-irradiated mice and increased as accumulated doses increased (Figure 1). Dose-response relationship between the incidences of dicentrics and centric rings and total accumulated doses up to 8000 mGy at both dose rates of 200 mGy/day and 20 mGy/day were obtained respectively. The dose-response curve for both 200 mGy/day and 20 mGy/day were almost linear up to 8000 mGy, indicating that these aberrations increased with accumulated dose for both dose rates.

The rate of chromatid-type aberrations also slightly increased with the accumulated dose of irradiation of 200 mGy/day and 20 mGy/day (Figure 1). The Equation obtained is shown in Figure 1 for 20 mGy/day and 200 mGy/day. The value of 0.086 at 0 Gy was obtained as the mean number of aberrations of dicentric and centric rings per 100 cells from the observation of 25 non-irradaiated 8-week-old mice at the beginning of irradiation.

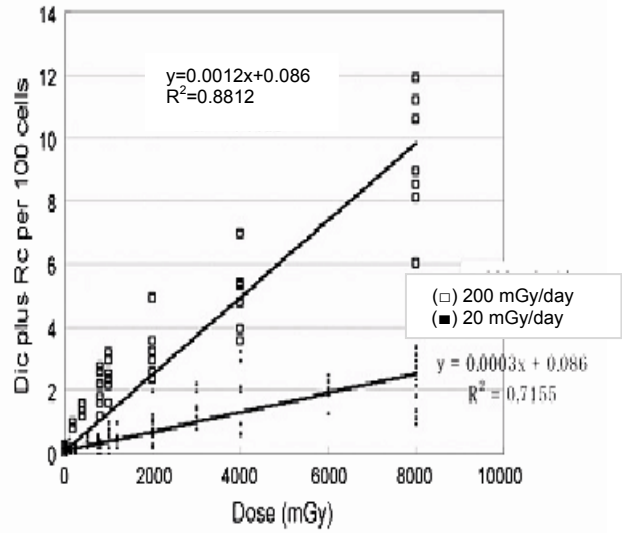

Figure 1. Frequencies of dicentrics and centric rings from mice after irradiation of less than 8000 mGy at LDR of 20 mGy/day (■) and 200 mGy/day (□). Each dot indicates the value obtained from individual mice.

The incidence of aberrations as dicentric and centric ring chromosomes were compared at two different dose rates (200 mGy/day and 20 mGy/day) at several identical total doses (Figure 1). The ratios of aberration frequencies between the two different dose rates at total doses of 8000 mGy, 4000 mGy, 1000 mGy and 800 mGy were 4.15, 2.78, 4.01, 5.06, and 6.58, respectively. Clear dose-rate effect was observed within these two different dose rates in the frequencies of dicentrics and centric rings. The present study revealed dicentric and centric ring aberrations in splenocytes continued to increase almost linearly up to 8000 mGy at dose rates of 20 mGy/day and 200 mGy/day as accumulated total doses rates increased. Dicentric chromosome aberrations from peripheral blood lymphocytes and *Dlb-1* mutations induced in the small intestine in mice showed no statistical difference in the dose responses between three different doses rates (550 mGy/day, 185 mGy/day and 64 mGy/day) as described by Sen *etal* (1990). Inverse radiation dose-rate effects have been seen for somatic and germ line mutations and DNA damage (Tucker *et al* 1998). Dose-rate effects on mutations rates in the mouse spermatogonia and spermatid were observed at the dose rates of 900 mGy/min to 0.8 mGy/min (1056 mGy/22 h/day), but not as much lower dose rate of 0.007 mGy/min (9.24 mGy/ 22 h/ day) as reported by Vilenchik *et al* (2000). Since the dose rate of 20 mGy/day (0.91 mGy/min when calculated for 22 h in a day) is approximately 20-200 times lower than those used in most other laboratories to observe adaptive response and dose-rate effects on mutation (Russel and Kelly, 1982).

The results obtained will be valuable for risk assessment of low-dose radiation. Splenocytes proliferated by ConA and LPS stimulation are considered to be mostly T- and B-lymphocytes, respectively. Lymphocytes with unstable-type aberrations could be eliminated in each cell division during long-term exposure to LDR γ – rays. It is possible that spleen is a reservoir for lymphocyte populations whi y could migrate into the spleen from other tissues incl ling the bone marrow, thymus, and lymph nodes of mice continuously irradiated for a long period. Another possibility is that a long-lived lymphocyte subset may be relevant for the linear increase of dicentric and centric ring chromosomes. The directly observed chromosome aberration rates of dicentric and centric rings between dose rates of 20 mGy/day and 200 mGy/day were compared at several doses to identify the existence of any dose-rate effect.

Chromosome instability detects by unstable-type aberrations

Tables 1 and 2 indicate that the mean percentage of chromosome aberrations in bone marrow cells and spermatocytes induced in irradiated mice has increased after LDR irradiation in comparison with non-irradiated mice.

On the other hand, the complexity of chromosomal aberrations in terms of number of unstable-type aberrations per cell will be a suitable cytogenetic index for evaluating chromosome instability after prolonged LDR irradiation.

Chromosomal aberrations such as Dic, Rc, Ra, Fg, min and del were scored and the total number of aberrations per cell was calculated using only aberrant cells in each mouse. Complexity was determined as a total number of unstable-type aberrations (Dic + Rc + Ra + Fg + min + del) divided by a total number of abnormal cells with chromosome-type aberrations. The results are summarised in Figure 2a and 2b. Their complexity started to increase from more than 200 mGy in the irradiation at 200 mGy/day and from more than 500 mGy in the irradiation at 20 mGy/day, and both increase from early days, 8 days and 1 day after initial irradiation, respectively (Figure 2b). On the other hand, our preliminary observation of translocation in spleen calls from mice with prolonged irradiation at 20 mGy/day showed that the complexity increased after 200 days after initial irradiation (more than 4000 mGy). The number of aberrations per cell was not different between unstable-type aberrations and translocations. Chromosome complexity was also compared at the

same irradiation period within 40 days between dose rates of 20 mGy/day and 200 mGy/day (Figure 2b). Complex aberrations were enhanced by irradiation with a dose rate of 200 mGy/day than 20 mGy/day indicating an influence of dose rate on chromosome instability.

Table 1. Percentage of chromosomal aberrations in bone marrow from mice irradiated with LDR at 20 mGy/day.

Dose [mGy/day]	No. of mice	Number of observed metaphases in cells	Number of observed metaphases with chromosomal aberrations	Percentage of metaphases with chromosomal aberrations (mean±S.E.)	Significance P <
0	5	296	14	4.74±0.36	-
250	5	250	19	9.5±0.5	P < 0.01
5000	5	250	45	22.3±0.63	P < 0.001
6000	4	200	59	23.9±1.33	P < 0.001
8000	4	200	69	27.8±1.52	P < 0.001

Table 2. Percentage of chromosomal aberrations in spermatocytes from mice irradiated with LDR at 20 mGy/day.

Dose [mGy/day]	No. of mice	Number of observed metaphases in cells	Number of observed metaphases with chromosomal aberrations	Percentage of metaphases with chromosomal aberrations (mean±S.E.)	Significance P <
0	5	220	7	3.51 ± 0.61	-
250	5	200	16	10.5 ± 0.9	P < 0.01
5000	5	200	25	12.7 ± 0.84	P < 0.001
6000	4	160	39	19.8 ± 1.03	P < 0.001
8000	4	160	42	21.6 ± 1.16	P < 0.001

On the other hand, the incidences of hyperploidy and complexity of chromatid-type aberrations which are determined as total number of ctg, ctb and exc divided by a total number of abnormal cells with chromatid-type aberrations did not increase with accumulated dose, although these two types of chromosome aberrations have been used as suitable markers for establishing chromosome instability (Farooqi and Kesavan, 1993).

It can be noticed that cells with heavy DNA damage may be eliminated by apoptosis whereas cells able to survive after exposure may have a higher probability of developing a mutation, and cells surviving after ionising radiation lead to genomic (chromosome) instability. Radiation-induced genomic instability arising at a large stage is demonstrated by increased rates of mutation, decreased cell survival, and various chromosome aberrations. An acute low dose to mice *in utero* induced high incidences of chromatid breaks and fragments and polyploidy cells in bone marrow cells after birth (Devi and Hossain, 2000a)]. Persistent chromatid-type aberrations were commonly observed in cells long-term cultured from haematopoetic and solid mouse tissues exposed to γ – rays (Devi and Hossain, 2000b and Watoson *et al* 2001). Lung cells from rates injected with superoxide dismutate showed a reduced number of micronuclei, which indicates the involvement of oxygen radicals in chromosome instability (Turker *et al.* 2004). Cells with chromatid-type aberrations result in one chromosomal deletion and one normal cell at the next cell division. Since unstable-type chromosome aberrations such as dicentric chromosomes are unstable and do not persist through the next cell cycle, a high percentage of these aberrations occurring by irradiation are eliminated after many cell generations. Radiation-induced chromosome or genetic instability has been demonstrated mostly with the use of accurate irradiation at high-dose rates (Cai and Cherian, 2001). Few studies have addressed whether low-dose radiation LDR can induce chromosome or genomic instability long-term after irradiation (Osipov *et al,* 2004). In this connection (Abrahmsson-Zetterberg *et al*, 2000) reported that exposure to radiation within a dose rate of 20 – 2000 mGy/h of mouse embryos showed no increase of chromosome instability at 35 days after birth, which indicates that LDR radiation doses might permit sufficient DNA repair. However, the present observation of exposure to 20 mGy/day

(0.91 mGy/h), which is much lower than reported by Nakano et al (2007), clearly showed chromosome instability.

From these experiments it was concluded that unstable-type aberrations, chromatid-type aberrations, and polyploidy cells are likely to be suitable markers for detecting chromosome instability *in vivo* after long-term LDR radiation, although the precise classification of direct radiation-induced aberrations and delayed chromosome aberrations could be difficult. Thus, the complexity of unstable-type and stable-type chromosome aberrations will also be used as a suitable cytogenetic marker of chromosome instability.

Moreover, it can be concluded that cells with chromosome instability can persist for a long period after irradiation and some form a benign or malignant clone which may develop into neoplastic transformation implicating the biological significance of continuous long-term exposure to LDR radiation. Information on the incidence of chromosome aberration frequencies and chromosome instability in persistent translocations will become more important for low-dose radiation-induced risk in humans.

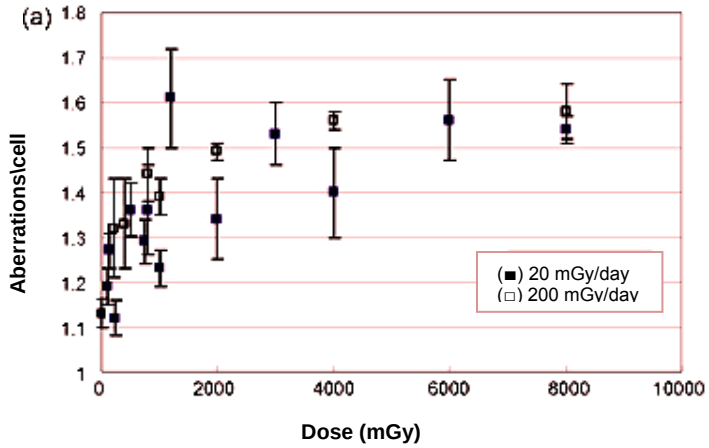

Figure 2a. Chromosome complexity (total number of unstable-type (Cu) aberrations per cell in abnormal cells) from mice after irradiation of less than 8000 mGy at LDR of 20 mGy/day (■) and 200 mGy/day (□). Each value indicates the mean ± standard error (S.E.) of data from at least seven mice.

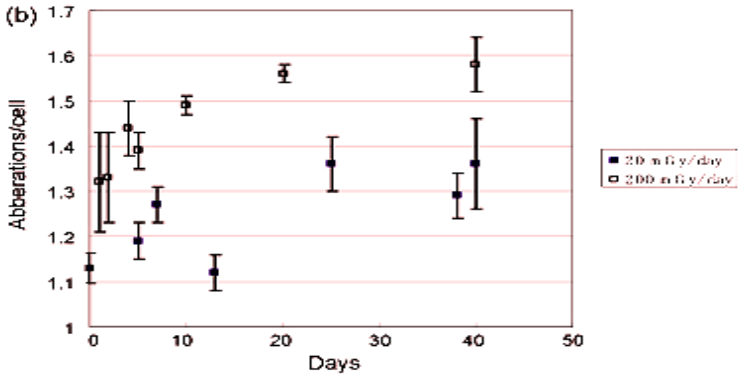

Figure 2b. Chromosome complexity (total number of unstable-type (Cu) aberrations per cell in abnormal cells) from mice after irradiation for 40 days at LDR of 20 mGy/day (■) and 200 mGy/day (□). Each value indicates the mean ± standard error (S.E.) of data from at least seven mice.

Corresponding author
ABDELMOHSEN M. SOLIMAN*
Therapeutic Chemistry Dept. National Research Centre, El-Behoos St., Dokki, Cairo, Egypt. P.O.Box: 12622
*solimanmohsen@yahoo.com

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