
Chronic exposure to low-dose of X-rays induces DNA double-strand breaks in mammalian cells

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Abstract: The purpose of present study is to evaluate the induction of clastogenic effect after chronic exposure to low dose of ionising radiation in both CHO-K1 cells and in their DNA Double-Strand Breaks (DSBs)-repair deficient mutant xrs-5. Cells were cultured for 14 dilution cycles and grown to confluency. Radiation treatment was performed once per cycle with 10 mGy X-rays to carry out the structural chromosome aberration test. The results showed a significant increase in the frequency achromatic lesions ($p < 0.001$) in CHO-K1 cells. In addition, xrs-5 cells showed an increase of chromatid and chromosome-type aberrations ($p < 0.001$). Sequential exposure produced a level of DNA damage that is amplified through successive cycles triggering the efficient repair of DSBs. The increased damage in xrs-5 cells provides evidence that the non-homologous end-joining repair of DNA DSBs is involved after low dose X-ray exposure.

Keywords: chronic exposure; serial effect of radiation; low dose radiation; X-ray; DNA damage; structural chromosome aberration test; mammalian cells; repair-deficient mutant cells.

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1 Introduction

Ionising radiation causes energy deposition (physical process) in living matter. This gives rise to direct damage in molecules (such as DNA and proteins) as well as to indirect damage through the production of radicals and reactive oxygen species (through radiolysis of water) initiating numerous radiochemical processes. For acute exposure, above a few Gy from radiation of low Linear Energy Transfer (LET), cell killing occurs as a result of the radiation-induced damage. Even if after exposure at a low dose-rate, cell killing is less significant, individual cells undergo some damage. Even at low dose-rate exposure, the relationship between direct and indirect damage induced by ionising radiation does not change. However, there are profound changes in the possibility of cells to scavenge or inactivate damaging radicals (cellular antiradical and antioxidant defences) and to eliminate damage induced in DNA by the various genetically controlled and enzymically driven (Averbeck et al., 2010). At low dose-rate exposure, a much smaller number of DNA Double-Strand Breaks (DSBs) are produced (by direct ionisation or by indirect attack from radiation-induced water radicals) and/or by the processing of multiple single lesions produced in close proximity on opposite DNA single strands (Lehnert, 2007). Rothkamm and Löbrich (2003) detected that doses as low as 1.2 mGy induce DSBs. Thus, DNA damage induced may be of biological relevance in possibly constituting pre-mutagenic lesions if not removed by efficient DNA repair. Moreover, experimental results obtained during the last ten years seem to indicate that genetic damage by low-dose of ionising radiation can be higher than expected due to phenomena such as bystander effect and genomic instability (Mothersill and Seymour, 2004; Preston, 2004; Little, 2006; Prise and O'Sullivan, 2009). A number of studies have demonstrated that low-dose radiation may elicit an adaptive radiation response (Olivieri et al., 1984; Wolf, 1998; Sasaki et al., 2002; Matsumoto et al., 2007; Grillo et al., 2009).

Newly emerging insight into the mechanistic basis of carcinogenesis supports the concept that the genetic effects of low-dose radiation cancer risks are considerably more complex than one might imagine based on linear no-threshold extrapolations from the high-dose radiation (Hagelstrom et al., 2008). Numerous processes affect low-dose radiation response. The DNA Damage Response (DDR) as well as the cellular consequences of low-dose radiation should be considered. A crucial question is whether the efficiency of enzymatic repair processes is high enough to let cellular functions after low-dose irradiation return to normal (Mullenders et al., 2009). If the repair is unsuccessful, incomplete or imprecise, the cell may die or may suffer alteration of genetic information. Several DNA repair mechanisms exist capable to remove pre-cytotoxic, pre-mutagenic

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and pre-clastogenic lesions. Molecular studies have shown that there are two main repair pathways which can repair radiation-induced DSBs, Non-Homologous End-Joining (NHEJ) and homologous recombination (HR) (Bedford and Dewey, 2002; Wynman and Kannar, 2006). It is well known that dysfunction of any protein in these processes leads to increased cell killing after irradiation because of the defect in DSBs repair (Grawunder et al., 1998; Thacker and Zdzienicka, 2003). Radiation sensitive mutant cell lines have been widely used in the analysis of repair processes and to get an understanding of the mechanisms that underlie genetic changes. One of them, *xrs-5* is a radiation-sensitive mutant derived from CHO-K1 cells and shows defects in DSB repair as well as in V(D)J recombination. The fully functional DSB repair pathway includes, among other components, the Ku70 and Ku80, DNA-PK, XRCC4 and ligase IV proteins (Li et al., 1995).

Most experimental data for ionising radiation-induced DSBs and their repair have been obtained after single and high-dose low-LET exposure where single cells are affected by several photons. Risk estimates for low dose are based on extrapolations from existing high-dose data and it is assumed that cellular systems including DNA repair operate with equal efficiency at low- and high dose. However, recent molecular and cellular studies suggest that this is not quite true (Mullenders et al., 2009). Low-dose radiation research is extremely relevant for public health, since humans in their homes and natural environment usually encounter very low dose. However, little information on the possible health risks of low-dose and low dose-rate exposures is available. Thus, studies of chronic exposures on human populations are important for the evaluation of genetic damage and carcinogenic risks (Carrano and Natarajan, 1988; Hagelstrom et al., 1995; Güerci et al., 2006). However, there some confounding factors to be considered that make it difficult to estimate low-dose radiation health risks. Consequently, it is not easy to elucidate low-dose radiation effects and underlying mechanisms. Thus, it would be important to develop an *in vitro* model to assess the induction of genetic damage and the mechanisms involved in controlled conditions.

This paper describes a cytogenetic analysis carried out after sequential (fractionated) low-dose X-ray exposure in both DSB-repair proficient cells (CHO-K1) and deficient (*xrs-5*) mutant Chinese hamster cells.

2 Materials and methods

2.1 Cell culture

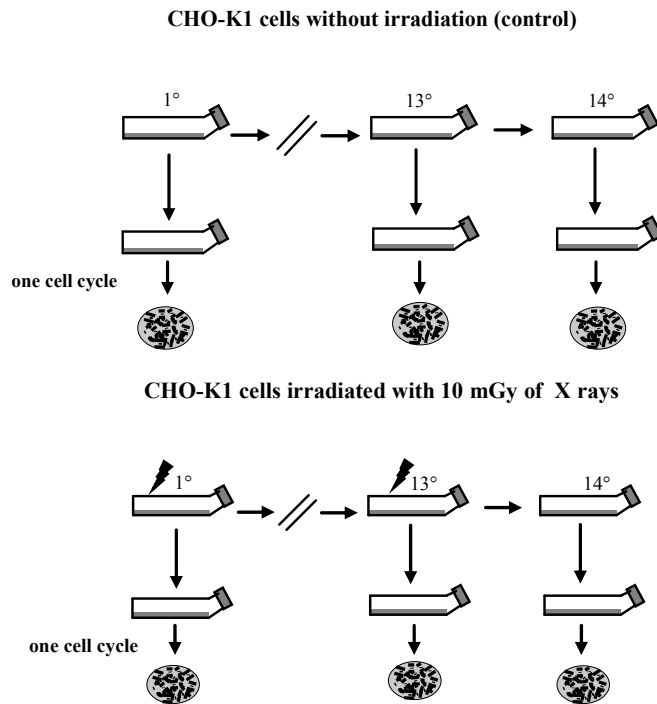
Chinese Hamster Ovary wild-type (CHO-K1) cells and CHO repair deficient mutant (*xrs-5*) lines were originally obtained from Department of Radiation Genetics and Chemical Mutagenesis, State University of Leiden, the Netherlands. Mycoplasma-free cells were grown in Ham F10 medium (Gibco-BRL, Los Angeles, CA, USA) supplemented with 10% inactivated foetal calf serum (Natocor, Carlos Paz, Córdoba, Argentina), 50 IU/ml of penicillin and 50 µg/ml of streptomycin sulphate at 37°C in a humidified atmosphere with 5% CO₂. Cells were cultured in Falcon T-25 Flasks, with 10 ml of culture medium. Cell viability was checked using the exclusion trypan blue (Sigma, St. Louis, MO, USA) method, for all cases viability was higher than 95%.

These cell lines have been frequently used by several researchers (Darroudi et al., 1990; Kashino et al., 2004; Leatherbarrow et al., 2006). Xrs-5 is one of a group of radiosensitive CHO cell lines originally isolated from an ethyl methanesulphonate treated culture of CHO-K1 cells (Jeggo and Kemp, 1983). The xrs-5 cells have a defect in DSBs rejoining and also differ from wild type CHO-K1 cells in various aspects of chromosome structure and nuclear morphology (Yasui et al., 1991; Schwartz et al., 1992; Dahm-Daphi et al., 1993).

2.2 Irradiation experimental procedure

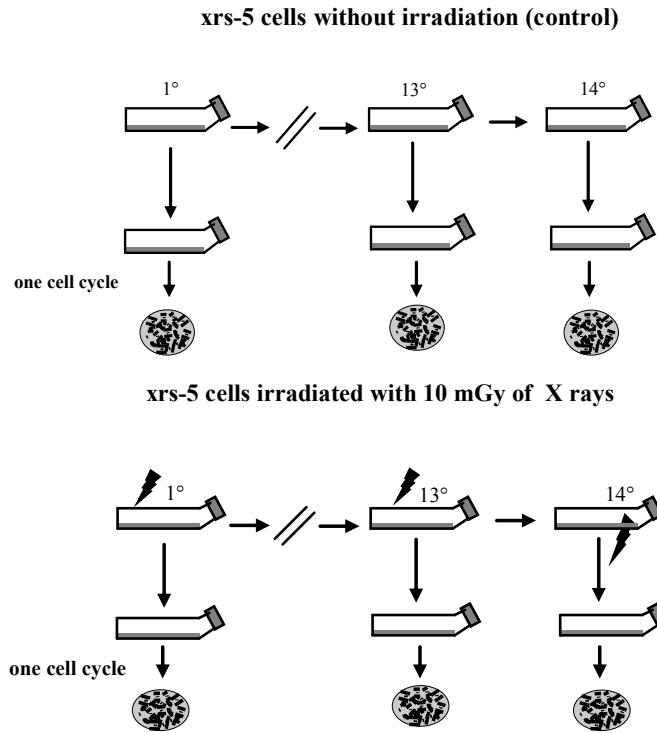
CHO-K1 and xrs-5 cells were cultured for 14 passages. Irradiation treatment with X-rays was performed once per passage when the cells were 90–95% confluent. For all the experiments, cells were washed twice with Phosphate-buffered Saline (PBS) and irradiated in this solution at room temperature. After treatment, cells were trypsinised and re-suspended in fresh medium. At each point of the serial procedure, the culture was diluted 1:2, one fraction was used to follow with chronic radiation and the other was used to carry out the structural chromosome aberration test. A control group (untreated cells) was incorporated in each cycle of irradiation (Figure 1).

Figure 1 Schematic diagram of irradiation experimental procedure. CHO-K1 and xrs-5 cells were cultured for 14th passages. Irradiation treatment with 10 mGy X-rays was performed once per passage. At each point of the serial procedure, the culture was diluted 1:2, one fraction was used to follow with chronic radiation and the other was used to carry out the structural chromosome aberration test



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Figure 1 Schematic diagram of irradiation experimental procedure. CHO-K1 and xrs-5 cells were cultured for 14th passages. Irradiation treatment with 10 mGy X-rays was performed once per passage. At each point of the serial procedure, the culture was diluted 1:2, one fraction was used to follow with chronic radiation and the other was used to carry out the structural chromosome aberration test (continued)



The irradiation dose employed was 10 mGy taking into account the dosimetry reported for previous investigations in our laboratory (Güerci et al., 2004; Güerci et al., 2005; Güerci and Grillo, 2007) and epidemiological studies (Barquinero et al., 1993; Paz-y-Miño et al., 1995; Balakrishnan and Rao, 1999; Heimers, 2000; Cavallo et al., 2002). The dose rate was 1.7 mGy/s. The X-ray apparatus used was a DSJ (Dental San Justo SA, Buenos Aires, Argentina) operated at 65 kV and 5 mA. Dosimetry was carried out with Keithley digital 35617 EBS microchamber PTW N 2336/414 (C-Com Industries, Robertville, MO, USA).

2.3 Structural chromosome aberration test

This test was carried out on the first post-irradiation metaphases. The lapse between radiation and fixation was 15–16 hours. Colchicine (Sigma) (0.1 µg/ml, final concentration) was added to all cultures 2 hours before fixation. Air dried slides were prepared, following routine protocols by using stained with Giemsa (Tjio and Levan, 1956). The experiments were performed twice in independent trials in order to assess reproducibility. A total of 300 metaphases per treatment (150 per repetition) were scored in coded slides. Statistical analysis was performed using the χ^2 test.

On the other hand, during cytogenetic analysis three types of aberrant metaphases were found:

- 1 with achromatid-type aberrations (gaps)
- 2 chromatid-type aberrations (chromatid and isochromatid breaks and chromatid exchanges)
- 3 chromosome-type aberrations (fragments, dicentric and ring chromosomes).

3 Results

The results obtained from the analysis of structural chromosomal aberrations in CHO-K1 cells treated with 10 mGy of X-rays for 14 cycles of irradiation are shown in Table 1. As expected (Güerci et al., 2004), ionising chronic radiation induced a significant increase in the frequency of abnormal metaphases when achromatic lesions are considered ($p < 0.001$) in relation to unirradiated cells. In the same way, significantly increased abnormal metaphases in xrs-5 irradiation cells in relation to untreated cells were found ($p < 0.001$). However, in this case, a significant increase of the frequency of the different types aberrations analysed, including metaphases with multiple aberrations, was found (Table 2).

Table 1 Frequencies of structural chromosome aberrations in CHO-K1 cells treated with 10 mGy of X-ray during 14 irradiation cycles. Standard error of mean is indicated between brackets

Irradiation cycles	Treatment	Abnormal metaphases (%) ¹	Chromosomal aberrations per 100 cells				
			AL ²	B ³	B ⁿ ⁴	RB ^r ⁵	DIC ⁶
1	Control	0.66	0.6 (0.08)	–	–	–	–
	Irradiation	1.00	1.0 (0.10)	–	–	–	–
2	Control	0.33	0.3 (0.06)	–	–	–	–
	Irradiation	1.66	1.3 (0.11)	–	0.6 (0.08)	–	–
3	Control	0.66	0.6 (0.08)	–	–	–	–
	Irradiation	2.00	2.0 (0.14)	–	–	–	–
4	Control	0.66	0.6 (0.08)	–	–	–	–
	Irradiation	2.04	2.4 (0.15)	–	–	–	–
5	Control	0.33	0.3 (0.06)	–	–	–	–
	Irradiation	2.33	1.6 (0.12)	0.3 (0.06)	0.3 (0.06)	–	–

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Table 1 Frequencies of structural chromosome aberrations in CHO-K1 cells treated with 10 mGy of X-ray during 14 irradiation cycles. Standard error of mean is indicated between brackets (continued)

Irradiation cycles	Treatment	Abnormal metaphases (%) ¹	Chromosomal aberrations per 100 cells				
			AL ²	B ³	B'' ⁴	RB' ⁵	DIC ⁶
6	Control	0.50	0.5 (0.06)	–	–	–	–
	Irradiation	3.00	2.6 (0.16)	0.3 (0.06)	–	–	–
7	Control	0.66	0.3 (0.06)	0.3 (0.06)	–	–	–
	Irradiation	3.33	3.0 (0.17)	0.6 (0.08)	–	–	–
8	Control	0.66	0.6 (0.08)	–	–	–	–
	Irradiation	3.66	3.6 (0)	0.3 (0.06)	–	–	–
9	Control	–	–	–	–	–	–
	Irradiation	6.09	4.3 (0.20)	1.7 (0.13)	–	–	–
10	Control	0.66	0.3 (0.06)	0.3 (0.06)	–	–	–
	Irradiation	2.33	2.3 (0.15)	–	–	–	–
11	Control	0.66	0.6 (0.08)	–	–	–	–
	Irradiation	3.66	3.3 (0.18)	0.3 (0.06)	0.3 (0.06)	–	–
12	Control	0.33	0.3 (0.06)	–	–	–	–
	Irradiation	3.66	3.0 (0.17)	0.3 (0.06)	–	0.3 (0.06)	–
13	Control	1.00	1.0 (0.10)	–	–	–	–
	Irradiation	3.23	3.2 (0.18)	–	–	–	–
14	Control	0.66	0.6 (0.08)	–	–	–	–
	Irradiation	4.17	3.0 (0.17)	0.4 (0.06)	0.4 (0.06)	–	0.4 (0.06)

Notes: ¹Abnormal metaphases: metaphases with at least one chromosomal aberration. Metaphases exhibiting only achromatic lesions were scored as abnormal; ²AL: Achromatic lesions; ³B': Chromatid breaks; ⁴B'': Isochromatid breaks; ⁵RB': Chromatid exchanges; ⁶DIC: Dicentric chromosomes.

Table 2 Frequencies of structural chromosome aberrations in xrs-5 cells treated with 10 mGy of X-ray during 14 irradiation cycles. Standard error of mean is indicated between brackets

Irradiation cycles	Treatment	Abnormal metaphases (%) ¹	Chromosomal aberrations per 100 cells						
			AL ²	B ³	B ^{m4}	RB ⁵	Frag ⁶	DIC ⁷	MA ⁸
1	Control	0.66	0.6 (0.08)	–	–	–	–	–	–
	Irradiation	2.66	–	0.6 (0.08)	1.0 (0.10)	–	0.6 (0.08)	–	0.3 (0.06)
2	Control	0.66	0.6 (0.08)	–	–	–	–	–	–
	Irradiation	4.00	0.6 (0.08)	1.0 (0.10)	1.3 (0.11)	0.3 (0.06)	–	0.3 (0.06)	0.6 (0.08)
3	Control	0.33	–	0.3 (0.06)	–	–	–	–	–
	Irradiation	4.66	0.6 (0.08)	2.0 (0.14)	0.3 (0.06)	–	0.6 (0.08)	0.6 (0.08)	0.6 (0.08)
4	Control	1.00	–	0.3 (0.06)	0.3 (0.06)	–	–	–	0.3 (0.06)
	Irradiation	3.33	0.6 (0.08)	1.3 (0.11)	1.6 (0.12)	–	0.3 (0.06)	0.6 (0.08)	–
5	Control	0.66	0.6 (0.08)	–	–	–	–	–	–
	Irradiation	5.00	0.6 (0.08)	1.3 (0.11)	–	0.3 (0.06)	1.0 (0.10)	0.3 (0.06)	2.0 (0.14)
6	Control	1.66	1.3 (0.11)	–	–	–	–	–	0.3 (0.06)
	Irradiation	5.33	1.0 (0.10)	3.3 (0.18)	–	0.3 (0.06)	–	1.0 (0.10)	0.6 (0.08)
7	Control	0.33	0.3 (0.06)	–	–	–	–	–	–
	Irradiation	5.86	2.7 (0.16)	2.4 (0.15)	0.3 (0.06)	0.3 (0.06)	–	1.3 (0.11)	0.3 (0.06)
8	Control	0.66	–	0.3 (0.06)	–	–	–	–	0.3 (0.06)
	Irradiation	5.73	0.8 (0.09)	4.0 (0.20)	0.3 (0.06)	–	–	0.6 (0.08)	1.1 (0.10)
9	Control	1.04	1.0 (0.10)	–	–	–	–	–	–
	Irradiation	7.84	–	3.9 (0.19)	1.9 (0.14)	–	–	–	1.9 (0.14)
10	Control	0.33	0.3 (0.06)	–	–	–	–	–	–
	Irradiation	5.49	0.9 (0.09)	2.8 (0.17)	–	0.3 (0.06)	–	0.9 (0.09)	0.9 (0.09)
11	Control	1.33	0.6 (0.08)	–	–	–	–	0.3 (0.06)	0.3 (0.06)
	Irradiation	6.13	1.4 (0.12)	3.3 (0.18)	0.3 (0.06)	0.3 (0.06)	0.3 (0.06)	0.6 (0.08)	0.9 (0.09)

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Table 2 Frequencies of structural chromosome aberrations in xrs-5 cells treated with 10 mGy of X-ray during 14 irradiation cycles. Standard error of mean is indicated between brackets (continued)

Irradiation cycles	Treatment	Abnormal metaphases (%) ¹	Chromosomal aberrations per 100 cells						
			AL ²	B ³	B ^m ⁴	RB ⁵	Frag ⁶	DIC ⁷	MA ⁸
12	Control	1.33	0.6 (0.08)	0.3 (0.06)	–	0.3 (0.06)	–	–	–
	Irradiation	4.55	–	2.3 (0.15)	0.4 (0.07)	–	0.4 (0.07)	2.3 (0.15)	–
13	Control	1.00	0.3 (0.06)	0.3 (0.06)	–	–	–	–	0.3 (0.06)
	Irradiation	6.00	0.3 (0.06)	4.0 (0.20)	0.3 (0.06)	0.3 (0.06)	–	1.6 (0.12)	0.6 (0.08)
14	Control	0.66	–	0.6 (0.08)	–	–	–	–	–
	Irradiation	5.39	0.9 (0.09)	3.8 (0.19)	0.9 (0.09)	–	–	1.6 (0.12)	0.3 (0.05)

Notes: ¹Abnormal metaphases: metaphases with at least one chromosomal aberration. Metaphases exhibiting only achromatic lesions were scored as abnormal; ²AL: Achromatic lesions; ³B': Chromatid breaks; ⁴B^m: Isochromatid breaks; ⁵RB': Chromatid exchanges; ⁶Frag: Chromosome fragments; ⁷DIC: Dicentric chromosomes; ⁸MA: Multiple aberrations.

Comparative analysis about the effect of long-time exposure of the two cell lines used, considering abnormal metaphases without achromatic lesions, is illustrated in Figure 2. The figure shows that mutant sensitive cells exhibiting 9.52-fold more clastogenic damage than the wild-type cells. An analysis detailed of the three types of aberrant metaphases is shown in Figure 3. Metaphases with achromatid-type aberrations were more frequent than any other type of aberration induced after chronic treatment in CHO-K1 cells (Figure 3a). However, xrs-5 cells also show a significant increase of chromatid and chromosome-type aberrations (Figures 3b and 3c). In addition, in all cases we can see an increase and decrease of clastogenic effect through of irradiation cycles of the chronic treatment.

Figure 2 Comparative analysis of frequencies of abnormal metaphases in CHO-K1 (white bars) and xrs-5 (black bars) cells treated with 10 mGy of X-ray during 14th irradiation cycles

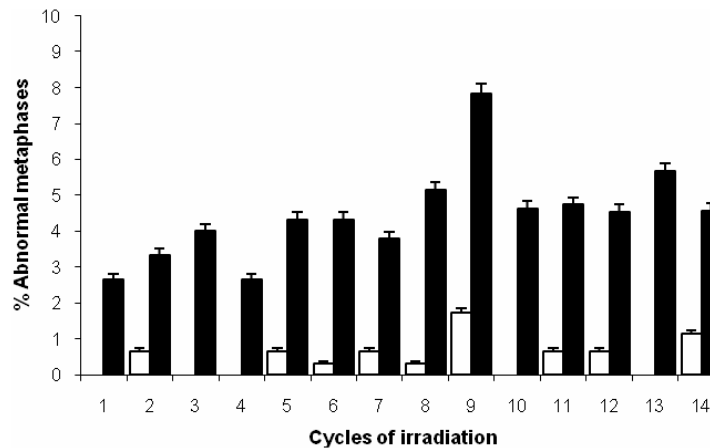
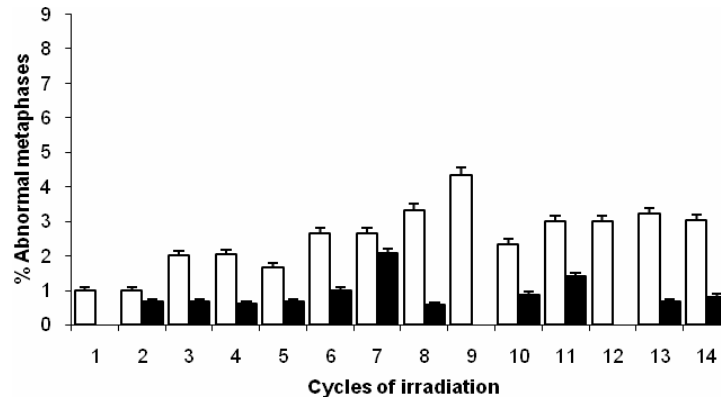
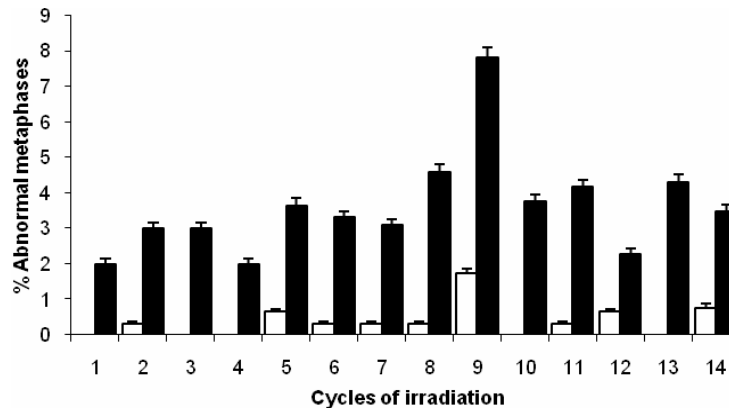


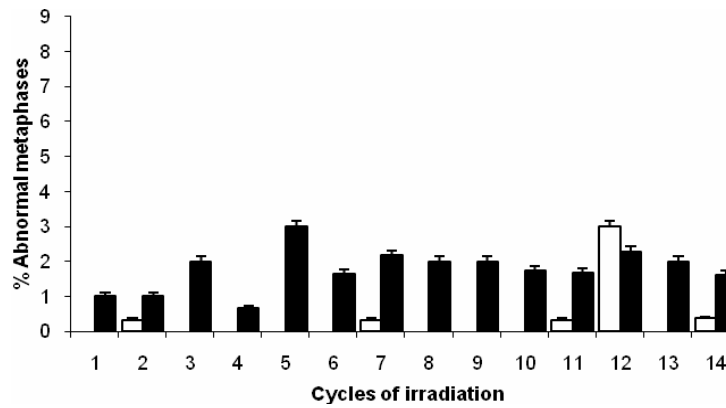
Figure 3 Frequencies of cells with different types of aberrations after treatment with 10 mGy of X-ray by 14th irradiation cycles. White bars, CHO-K1 cells; black bars, xrs-5 cells



(a) Achromatid-type aberrations



(b) Chromatid-type aberrations



(c) Chromosome-type aberrations

4 Discussion

The biological effects of radiation have been extensively studied throughout the 20th century. The nature of the lesions in DNA and other macromolecules are relatively well described (Jeggo and Lavin, 2009). However, the cellular response mechanisms at low-dose chronic exposure are not well established. Moreover, there is a need to investigate the efficiency of the damage response pathways and evaluate if the knowledge gained from studies with high radiation doses can be applied to low radiation doses. Consequently, it is very important to evaluate if the cell responds to DNA damage in the same way to low or high dose.

Ionising radiation induces various lesions arising in DNA that may promote mutagenic effects. The most potentially damaging lesions are DSBs, which contribute to genome instability or cell death (van Gent et al., 2001; Löbrich and Jeggo, 2007). DNA repair mechanisms have evolved to protect cells against DSB. Mutations in proteins that constitute these repair pathways determine the radiosensitivity status of the cell (Jeggo and Lavin, 2009). Furthermore, alterations in the heterochromatin and nuclear envelope also have been associated with radiation sensitivity (Schwartz and Vaughan, 1989; Gordon et al., 1990) and could be contributed with an increase of DNA damage (Yasui et al., 1991).

In the present study, we evaluated the cytogenetic effects in both CHO-K1 and in their DSB-repair deficient mutant *xrs-5* cells, sequentially exposed to low doses of X-rays. The results showed an increase of achromatid-type aberration after chronic irradiation in CHO-K1 cells, in accordance to previous cytogenetic studies that showed an increase of clastogenic damage after sequential radiation with 2.5–10 mGy range doses in mammalian cells (Güerci et al., 2005). This fact could be the result of base damage or Single Strand Breaks (SSBs) produced by an oxidative environment in G2 phase of cell cycle. Although it is likely that some DNA DSBs are also produced, most of these would be efficiently repaired. In this sense, Grudzenskia et al. (2010) presented evidence that an inducible response by oxidative radicals is required for efficient repair of DSBs in human fibroblasts, at low-doses treatment. Our model of chronic exposure could offer this environment to trigger repair of DSBs in CHO-K1 cells. On the other hand, in *xrs-5* cells low-dose radiation induced clastogenic damage was higher than in CHO-K1 wild-type cells. This fact could be attributable to a reduced capacity of *xrs-5* to repair DNA DSBs and to a higher level of initial damage attributable to different nuclear architecture in these two cell lines (Korte and Yasui, 1993).

Our results suggested the ability of low dose of X-ray (10 mGy) to induce DSBs, evidenced at the cytogenetic level by the appearance of chromosome-type aberrations in *xrs-5* cells treated at G0/G1 phase of cell cycle. In addition, a constant oxidative environment produced as consequence of sequential exposition and the presence of bystander factors could be the cause of DSBs in G2 phase evidenced as chromatid-type aberrations. These results are in relation to other studies that have shown that *xrs-5* cells presented a mixture of chromosome- and chromatid-type aberrations in mitosis when being irradiated in G1 after an acute radiation (Darroudi and Natarajan, 1987; Nagasawa and Little, 2002).

Additionally, a level of cyclic clastogenic damage in both cell lines during all the radiation cycles was observed. It could be explained through a different process, such as bystander effect and radioadaptive response, although induction of genomic instability cannot be ruled out. The increased damage could be referred to bystander response. Several researches have reported this effect in *xrs-5* cells in the cytogenetic and mutagenic assay, suggesting that DSBs and base damage are caused by factors secreted in the medium from irradiated cells (Nagasawa and Little, 2002; Kashino et al., 2007) and that doses as low as 5 mGy of low LET trigger bystander effect (Mothersill and Seymour, 2004). On the other hand, the reduced damaging effect could be determined by the extent of cell killing. However, our results showed that cellular viability, measured by trypan blue exclusion die, was not modified throughout the successive irradiations (data not shown), one of the explanations for these results could be that radioadaptive response is involved in chronic exposure to low dose of ionising radiation.

Our previous research have also shown a cyclical DNA damage measured by comet assay in CHO-K1 and *xrs-5* cells, but the percentage of DNA damage was higher than the observed in the analysis of CA (Güerci and Grillo, 2007). This fact can be attributed to the fact that the comet assay was performed immediately after radiation and detected the initial levels of DNA damage, and CAs represent the result of DNA damage after one cell cycle, which involves several processes, such as DNA repair and misrepair. Thus, it is evidenced that action of other repair mechanisms differs from NHEJ in response to low doses of ionising radiation.

The radiation doses that can potentially be encountered during a person's life range from a few mGy to several tens of Gy. However, many people will never encounter high radiation doses but will likely be exposed to doses in the mGy range, through radio-diagnostic examinations, X-ray scans as well as the annual dose received from natural sources (Grudzenskia et al., 2010), while taking into account occupational exposure. Thus, evaluation of risks at this low level of radiation is clearly of social relevance (Brenner et al., 2003).

The risk estimates available are based on epidemiological studies, mainly survivors of Hiroshima and Nagasaki. These studies have demonstrated a lineal increase with dose for moderate and high doses (>50–100 mGy) (Preston et al., 2004). Estimates below this level are difficult to obtain directly from epidemiological data. Nevertheless, the validity of such a lineal extrapolation into the dose range of a few mGy would require that the biological response is equally efficient after low and high doses (Goodhead, 2010). In this sense, Grudzenskia et al. (2010) confirmed data showed by Rothkamm and Löbrich (2003) that DSBs induced by low-dose are repaired at a slower rate than DSBs produced by higher doses. Thus, the efficiency of the repair of DSBs is different in the mGy range compared with high-dose, challenging one of the most important assumptions for risk estimates in the low-dose range, i.e. the LNT-hypothesis.

The final response to radiation chronic exposure could be determined not only by cellular repair processes but also by related cellular functions that optimise the opportunity for recovery of damage.

In summary, the results suggest that sequential exposure to low-dose, under these experimental conditions, induce clastogenic effect. The environmental oxidative produced could be the cause of achromatid-type aberrations and could also trigger response for efficient repair of DSBs. The finding of cytogenetic damage in *xrs-5* cells could be the

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evidence that NHEJ repair mechanism is involved in repair DNA damage induced by low-dose of X-ray. Additionally, the cyclical genotoxic damage suggests the possible induction of a complex phenomenon including different processes, such as genomic instability, bystander effects and radioadaptive response. However, further studies are necessary to confirm these assumptions.

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