

# High-LET-induced chromosomal damage: time-dependent expression

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## Abstract

Chromosome aberrations are routinely analysed in metaphase cells at one sampling time post-irradiation. Yet, accumulating evidence shows that radiation-induced cycle perturbations and mitotic delay influence the yield of aberrations detectable in mitosis. In extended time-course studies an drastic increase in the number of aberrations with sampling time has been observed after particle irradiation, while after the exposure to sparsely ionizing radiation a less pronounced effect has been found. This difference in the time-course of chromosomal damage is particularly important for the determination of accurate RBE values. As will be discussed, meaningful RBE values for particles can only be obtained, if cells are analysed at multiple sampling times and the complete time-course of aberrations is considered. Otherwise, particle-induced damage will be over- or underestimated. Moreover, depending on the cell system chosen for the analysis, factors like the loss of damaged cells due to apoptosis or a permanent cell cycle arrest complicate the determination of accurate RBE values based on chromosome data.

KEYWORDS: High LET radiation, RBE, time-course of chromosome aberrations, radiation risk assessment.

## 1. Introduction

Chromosome aberrations are regarded as the most sensitive biological indicator of radiation-induced damage and have been widely used over the past decades to estimate the risk associated with radiation exposure. Up to now the measurement of aberrations has been usually accomplished by the scoring of metaphase cells. According to the standard protocol the analysis of aberrations is confined to cells at the first post-irradiation mitosis collected at one sampling time. Yet, evidence is accumulating that radiation-induced cell cycle perturbations [1, 2] and mitotic delay [3, 5] play a crucial role for the interpretation of chromosome data [5, 6].

Extended studies investigating the effect of ionizing radiation on the time-course of aberrations revealed a correlation between the cell cycle delay and the aberration burden of a cell, i.e. heavily damaged cells have been found to reach mitosis later than undamaged or slightly damaged cells. For sparsely ionizing radiation which has only a minor effect on the cell cycle time [e.g. 3, 4], a slight increase in the aberration yield with time [3, 4, 7-11] or a homogeneous sensitivity of the cells has been reported [4, 12-14]. In contrast, for high LET radiation which causes drastic cell cycle perturbations [1, 2], a more pronounced increase in the aberration yield with sampling time has been observed [3-5, 11]. This difference in the expression of low and high LET induced chromosomal damage complicates the determination of meaningful RBE values. RBE values obtained from single fixation regimes will strongly depend on the interval chosen for the analysis, ranging from values below 1 at early sampling times up to values greater than 1 for late sampling times [3]. Yet, up to now the use of

multiple fixation regimes to estimate the amount of damage induced within the whole cell population is not common. Consequently, the low RBE reported for particles with LET values above 300 keV/ $\mu$ m [15] and references therein may be attributed to the use of only one, early fixation time so that drastically delayed heavily damaged cells have not been included in the analysis.

In the following experimental evidence is presented demonstrating that multiple fixation regimes are an indispensable prerequisite for a meaningful estimation of the RBE for the induction of aberrations. Then, by means of a mathematical approach [6, 16] the total amount of damage induced within the whole cell population can be determined and used for RBE calculation. In addition, confounding factors like the loss of damaged cells due to apoptosis or the restriction of the analysis to only one aberration type will be discussed.

## 2. Time-course of chromosome aberrations

### 2.1. Raw data

To illustrate the effects of high and low LET radiation on the time-course of aberrations, recent experiments performed at GSI (Darmstadt, Germany) with 3 established Chinese hamster cell lines (V79, CHO-K1 and xrs5) are summarized. In these studies cells were irradiated in G1-phase with X-rays or heavy ions (Ne, Ar, Kr, Au) with LET values in the range of 390 to 4000 keV/ $\mu$ m. Metaphase cells were collected at 5 to 12 subsequent harvesting times between 10 to 34 h after exposure. This time interval corresponds to almost 3 generation times of control cells and ensures that also very delayed cells

are included in the study. To confine the analysis to genuine first post-irradiation metaphases the fluorescence-plus-Giemsa (FPG) technique was applied (for experimental details see [3]).

These experiments show that after particle exposure in all cell lines studied the number of aberrations/cell increases drastically with sampling time and that this effect depends on LET [3-5 and Nasonova and Ritter, unpublished data]. In contrast, in X-irradiated V79 [3] and CHO-K1 cells [4] a slight increase in the frequency of aberrant cells and aberrations/cell has been found, while in xrs5 cells [4] no increase has been observed. For example, in V79 cells exposed to Ar ions (4.6 MeV/u, 1840 keV/ $\mu$ m) the aberration frequency increased between 14h and 26h post-irradiation by a factor of 20, but in X-irradiated cells only by a factor of 3 [3]. Generally, these effects are not restricted to established cell lines. A time- and LET-dependent expression of chromosomal damage has also been observed in cultured human lymphocytes [7-11] and human skin fibroblasts (Nasonova and Ritter, unpublished data) and can be related to differences in the spatial energy deposition of both radiation types. As described in [3] the exposure of cells to particles results in an inhomogeneous energy deposition per cell. The inhomogeneity is determined by different numbers of direct particle hits per cell nucleus and by the nonuniform dose distribution inside the particle track. Cells with a low number of particle traversals and correspondingly low chromosomal damage enter mitosis earlier than cells with a high number of hits and correspondingly more chromosomal damage. In contrast, when X-rays are applied, the energy is fairly uniformly deposited leading to a more homogeneous distribution of aberrations and delay times within the exposed cell population.

## 2.2. Mathematical analysis of data: determination of the total yield of damage

To account for the time-dependent expression of chromosomal damage, a mathematical approach was used [6, 16], which allows to quantify the amount of damage induced within the whole cell population. Briefly, the number of aberrant cells and aberrations obtained at each sampling time is weighted with the corresponding mitotic index. Then, the data are corrected for cell proliferation to account for the dilution of damaged cells at later sampling times due to the division of undamaged cells. Finally, the total yield of aberrations is determined by integrating the area under the yield-time curve. Dose-response curves generated for the total yields can then be used for the calculation of RBE values.

As an example, in figure 1 the dose-response curves for the total (integrated) amount of aberrations induced in V79 cells by various ions are plotted. Calculation of the RBE values as in reference [15], i.e. by dividing a dose of 4 Gy X-ray by the isoeffective dose of particles, yields for 10.6

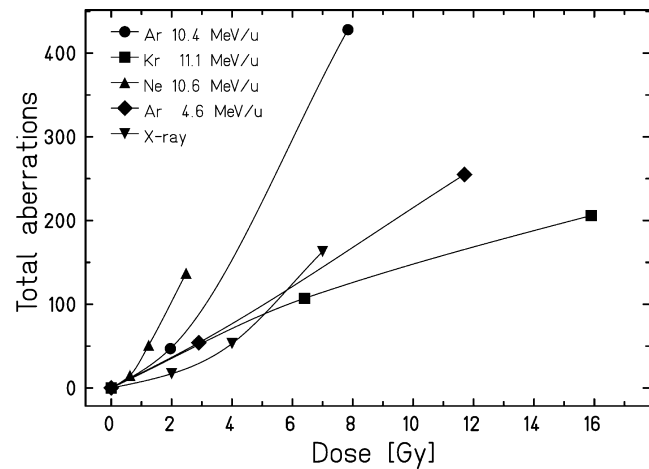


Fig. 1 – The total number of aberrations per 100 initial cells. V79 G1-phase cells have been exposed to 10.6 MeV/u Ne ions, 10.4 and 4.6 MeV/u Ar ions and 11.1 MeV/u Kr ions with LET values of 390, 1226, 1840 and 3980 keV/ $\mu$ m, respectively. Metaphase cells were collected at multiple sampling times and chromosomal damage was scored in first cycle cells following differential staining. Finally the total number of aberrations induced within the whole cell population has been determined as described in detail in [6].

MeV/u Ne ions (LET: 390 keV/ $\mu$ m), 10.4 and 4.6 MeV/u Ar ions (LET: 1226 and 1840 keV/ $\mu$ m) and 11.1 MeV/u Kr ions (LET: 3980 keV/ $\mu$ m) values of 3.2, 1.9, 1.4 and 1.3, respectively. Yet, if the analysis of data is restricted to the total (integrated) yields of dicentrics, which are regarded as the key aberrations in biological dosimetry [e.g. 7], much lower RBE values are obtained: For the ions listed above, they amount to 2.5, 1.25, 0.5 and 0.2, respectively. These differences between RBE values calculated for the total amounts of aberrations and dicentrics can be explained by the fact that the spectrum of aberrations changes, when LET increases. While after sparsely ionizing radiation the spectrum of aberrations is dominated by exchange-type aberrations like dicentrics, after high LET exposure breaks are preferentially formed. These alterations have been observed in different cell types and are regarded as a consequence of the qualitative different lesions produced by low and high LET radiation (for further details see [3, 5] and references therein). Thus, to obtain an accurate RBE value for the production of chromosome aberrations by heavy ions, all aberration types should be considered.

## 3. Influence of apoptosis and cell cycle arrest on the amount of scorable chromosomal damage

In recent years evidence is increasing that in some cell systems like human lymphocytes and human skin fibroblasts a significant proportion of the cell population does not reach the first post-irradiation mitosis at all, i.e. the damage carried by these cells cannot be detected by conventional cytogenetics. When human lymphocytes are used, cells are lost

due to apoptosis [e.g. 17,18], while in human skin fibroblasts a reduction in cell proliferation activity due to a permanent cell cycle arrest affects the number of scorable cells [19-21 and references therein]. One method to estimate the percentage of cells, which reach mitosis within a given time span, is the mathematical approach [6, 16] described in section 2.2. For example, application of this method to chromosome data obtained for human fibroblasts (Ritter and Nasonova, unpublished data) clearly shows that in these cells already low doses of low LET radiation reduces drastically the number of cells which progress to mitosis (see figure 2). In contrast, in other cell lines (e.g. V 79 cells) which are apoptosis-resistant and do not undergo a permanent cell cycle arrest, most cells reach mitosis even after high LET exposure (see figure 2) and thus can be analysed for chromosomal damage.

Both effects, the 'loss' of damaged cells due to apoptosis or permanent cell cycle arrest, can contribute to an underestimation of radiation-induced damage. The underestimation of cytogenetic damage as a result of apoptosis was recently reported by Guo et al. [22]: Investigations of several cell lines with similar survival but different apoptosis rates showed that in cell lines with a high rate of apoptosis the cytogenetic assay (here: micronucleus test) underestimates cell survival, while in apoptosis-resistant cell lines it predicts the survival rate fairly good. Similarly, the cell growth characteristics of human skin fibroblasts obtained from patients with acute and/or late side effects following radiotherapy was found to affect the evaluation of cytogenetic

damage: A good correlation between the expression of cytogenetic damage and radiation sensitivity of a donor have been found in normal growing cells but not in slow growing cultures [23]. Yet, further studies are needed to assess in more detail the influence of these effects on the expression of aberrations in metaphase cells.

#### 4. Summary

The low RBE values reported in the literature for the production of aberrations by particles [e.g. 15] may be attributed to the fact that damage was measured at only one post-irradiation sampling time and thus drastically delayed heavily damaged have been automatically excluded from the analysis. The use of multiple fixation regimes together with a mathematical analysis, which allows to determine the damage induced within the whole cell population, will result in more accurate RBE values. For these estimates all aberration types should be used and not only dicentrics, since the spectrum of aberrations changes with LET. Moreover, assessment of the relationship between radiation-induced apoptosis, permanent cell cycle arrest and the amount of chromosomal damage scorable in metaphase cells seems to be important to predict more accurately the radiation response.

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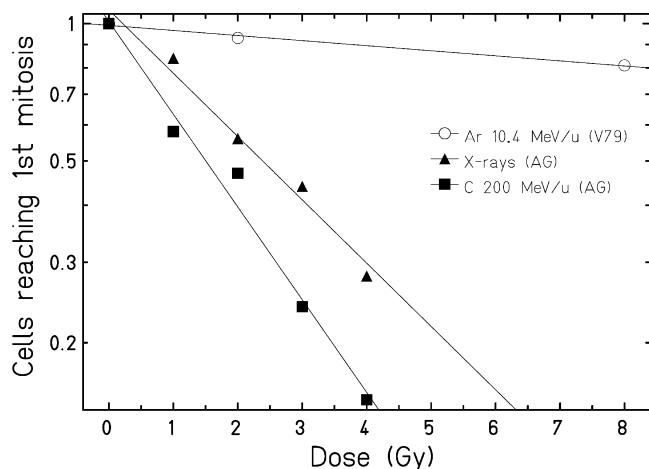


Fig. 2 – Estimated fractions of cells which reach the first post-irradiation mitosis, i.e. can be analysed for chromosomal damage. Human skin fibroblasts (AG 1522, closed symbols) have been exposed to X-rays and 200 MeV/u C ions (LET: 16 keV/ $\mu$ m) and metaphases were collected at 13 sampling times up to 70h post-irradiation. In the case of V79 Chinese hamster cells (open symbols) irradiation was performed with 10.4 MeV/u Ar ions (LET: 1226 keV/ $\mu$ m) and cells were collected at 12 sampling times up to 34h postirradiation. The raw data have been used to calculate the fraction of cells which were able to enter first mitosis as described in [6, 16].

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