Quantification of High LET Induced Chromosome Aberrations

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The classical cytogenetic assay relies on the scoring of chromosomal damage in cells at metaphase. According to the standard protocol the analysis is confined to cells at the 1st post-irradiation mitosis collected at one, early sampling time. For sparsely ionizing radiation this protocol allows a reasonable estimate of chromosomal damage, because in all commonly used cell system no or only a slight increase in the aberration yield with time has been observed [1,2 and references therein]. In contrast, as shown in our experiments with different Chinese hamster cell lines [e.g. 1-4] single fixation regimes do not necessarily allow a meaningful quantification of high LET induced cytogenetic damage. Following particle exposure a drastic increase in chromosomal damage with time has been observed. For example, in V79 cells exposed to X-rays the aberration frequency increased by a factor of 3 [1], but in Ar-irradiated samples by a factor of 20 (fig. 1). To account for the time-dependent expression of damage a mathematical approach was used [5], which allows to determine the total amount of aberrations induced within the whole cell population. Based on these total aberration yields RBE values have been calculated. For 10.4 MeV/u Ar (fig. 1) an RBE of 1.9 is obtained. Similarly, for V79 cells exposed to 10.6 MeV/u Ne ions (LET: 390 keV/μm) or 11.1 Kr ions (LET: 3980 keV/μm) RBE values of 3.2 and 1.3 are estimated. As expected, these RBE values are much higher than those reported in the literature [e.g. 6], because in our analysis also drastically delayed heavily damaged cells are included. Moreover, extension of these studies to human primary skin fibroblasts and lymphocytes which are usually used for radiation risk assessment in humans, have shown that the above described effects are not restricted to Chinese hamster cells. For example, in human lymphocytes exposed to 200 MeV/u Fe ions the aberration yield rises in 1st cycle cells by a factor of 7, while after X-irradiation only an increase by a factor of 1.2 is observed.

Furthermore, there is increasing evidence that besides the above described delay of heavily damaged cells additional factors might interfere with the expression of aberrations in metaphase cells. In the case of human lymphocytes apoptosis as well as interdonor variability seem to be important, while in the case of human fibroblasts a permanent cell cycle arrest in G_1 and/or G_2 might contribute to an underestimation of radiation induced damage. For example, as shown in figure 2, even low doses of low LET radiation reduce drastically the number of fibroblasts which are able to proceed to the 1st post-irradiation mitosis. In contrast, this effect is less pronounced for V79 cells which are "apoptosis-resistant" and do not undergo a permanent cell cycle arrest. Even after exposure to 6.5 Gy Kr ions (11.1 MeV/u, 3980 keV/μm) about 50% of V79 cells reach the 1st post-irradiation mitosis and thus can be analysed for chromosomal damage (see fig. 2).

Further experiments are in progress to examine the extent to which interdonor variability as well as high LET induced apoptosis or permanent cell cycle arrest affect the aberration yield detectable in metaphase cells.

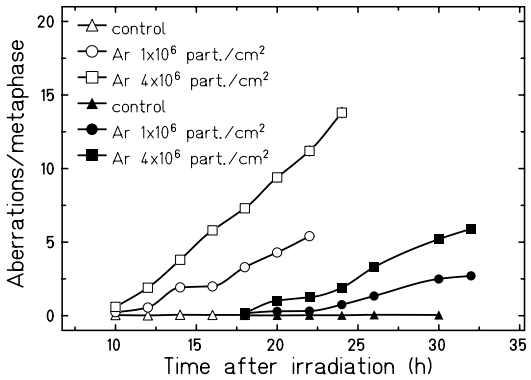

Figure 1: Time-course of aberrations in V79 cells after Ar-irradiation (10.4 MeV/u, 1226 keV/μm). Cells have been exposed in G_1 and chromosomal damage was scored at several sampling times (open symbols: 1st cycle metaphases; closed symbols: 2nd cycle cells). For further details see [3].

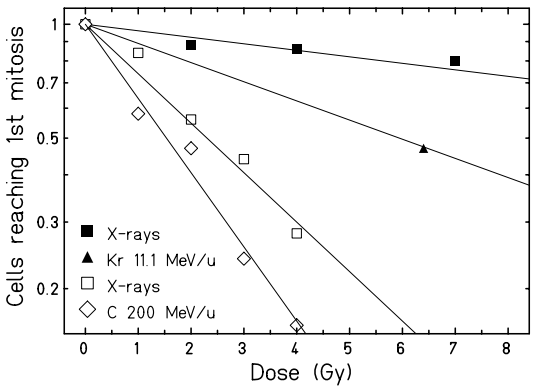

Figure 2: Fractions of human skin fibroblasts (open symbols) and V79 Chinese hamster cells (closed symbols) which reach the 1st post-irradiation mitosis. Human fibroblasts have been exposed to X-rays or 200 MeV/u C ions (LET: 16 keV/μm), V79 cells to X-rays or 11.1 MeV/u Kr ions (LET: 3980 keV/μm). Calculations were performed as described in reference [5].

References

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