

6 DNA-DSB IN CHO-K1 CELLS INDUCED BY HEAVY IONS: BREAK REJOINING AND RESIDUAL DAMAGE (GSI)

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DNA double strand breaks (DSBs) are the critical lesions involved in cellular effects of ionizing radiation. Therefore, the evaluation of DSB induction in mammalian cells after heavy ion irradiation is an essential task for the assessment of high-LET radiation risk in space.

Of particular interest has been the question of how the biological efficiency for the cellular inactivation endpoint relates to the initial lesions (DSBs) at varying LETs. For cell killing, an increased Relative Biological Efficiency (RBE) has been determined for high-LET radiation around 100-200 keV/ μm [3]. At higher LET, the RBEs decrease again to values below one for the very heavy particles. At GSI, DSB-induction was measured in CHO-K1 cells following irradiation with accelerated particles covering a wide LET range. The electrophoretic elution of fragmented DNA out of agarose plugs in a constant electrical field was applied for the detection of DSBs [5]. The fraction of DNA retained was determined considering the relative intensities of ethidium bromide fluorescence in the well and in the gel lane. Dose-effect curves were established, from which the RBE for DSB induction was calculated at a fraction of 0.7 of DNA retained.

RBE values are compiled in fig. 1, together with some literature values included for comparison. The data show RBEs between one and two up to an LET of 100 keV/ μm , followed by a steady decrease in RBE for higher LET values. In contrast to previously reported data [2], the yield of DSBs per unit dose does not increase in the LET region where enhanced cell inactivation is observed. Thus, the cellular endpoint is not related to induced DSBs directly but may rather depend on the fate of these lesions after processing in the cell. In order to gain information about the cellular capacity to cope with heavy ion induced strand breaks, rejoining of DSBs and residual DNA damage after repair incubation were investigated. For this purpose, CHO cells were incubated for various periods at 37 °C after irradiation with particle beams. In fig. 2 the effect of an increase in LET for one particle species is shown. Rejoining is dramatically impaired for the low energetic ions. The effect of increasing LET for higher Z particles is depicted in fig. 3.

In summary, these rejoining studies are in line with an enhanced severity of the DNA DSBs at higher LETs, resulting in a decreased repairability of the induced lesions. However, no information concerning the fidelity of strand breaks rejoining is provided in these studies. To assess correct rejoining of DNA fragments an experimental system involving individual DNA hybridization bands has been set up. In preliminary experiments, Sal I generated DNA fragments of 0.9 Mbp were irradiated with xrays and incubated for repair. However, restitution of the original signals was not observed, probably due to the high radiation dose necessary for breakage of a fragment of this size. A banding pattern with NotI hybridization signals in a higher MW range (3 Mbp) has been obtained by varying the electrophoretic conditions and correct rejoining studies will be further developed in this system.

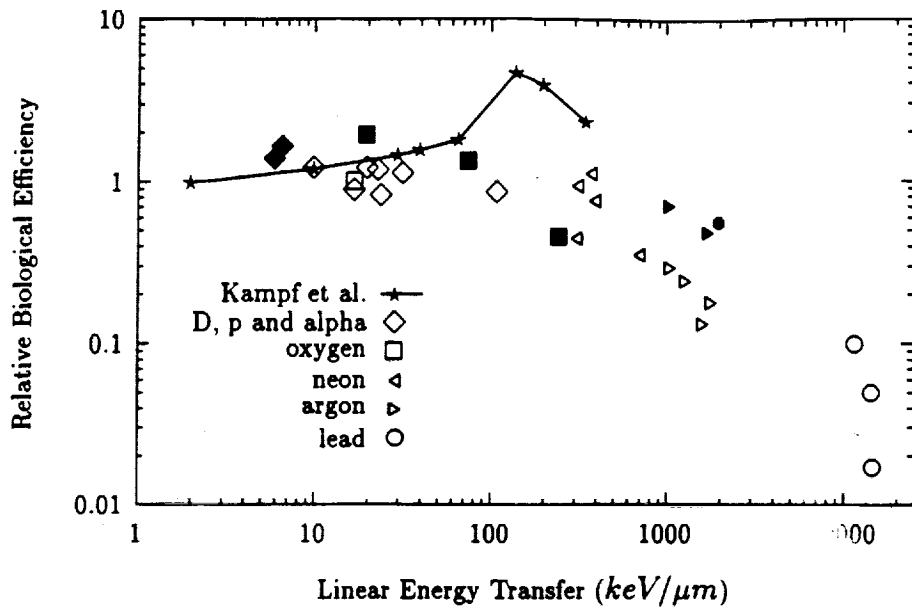


Figure 1: RBE values for the induction of DNA double strand breaks in mammalian cells. Own data are shown (closed symbols), together with results from others [1, 2, 4, 6]

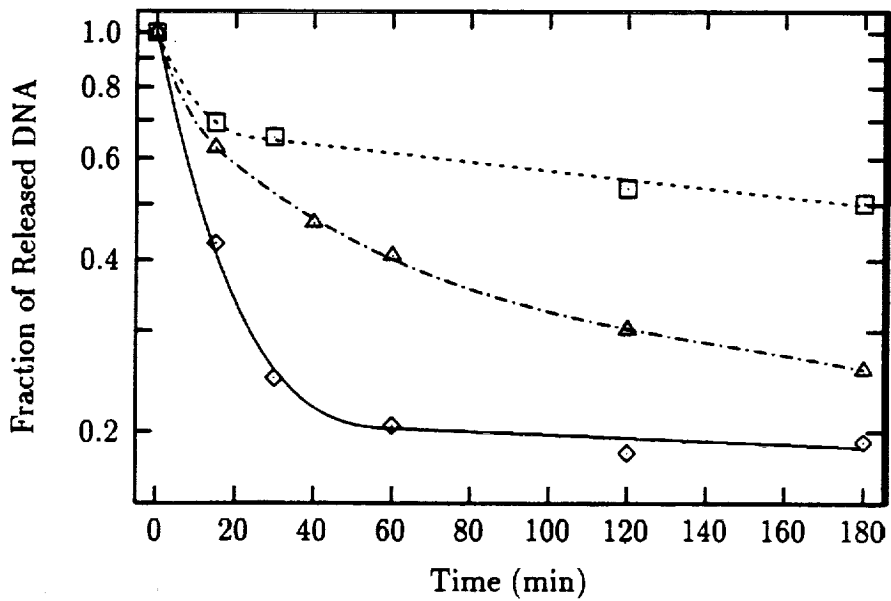


Figure 2: Rejoining of DNA double strand breaks in CHO-cells after irradiation with 250 kV X-rays (\diamond), 390 MeV/u neon-ions (\triangle) and 10 MeV/u neon-ions (\square). LETs correspond to 2 $\text{keV}/\mu\text{m}$, 30 $\text{keV}/\mu\text{m}$ and 370 $\text{keV}/\mu\text{m}$. With increasing LET, both kinetics and extent of rejoining decrease.

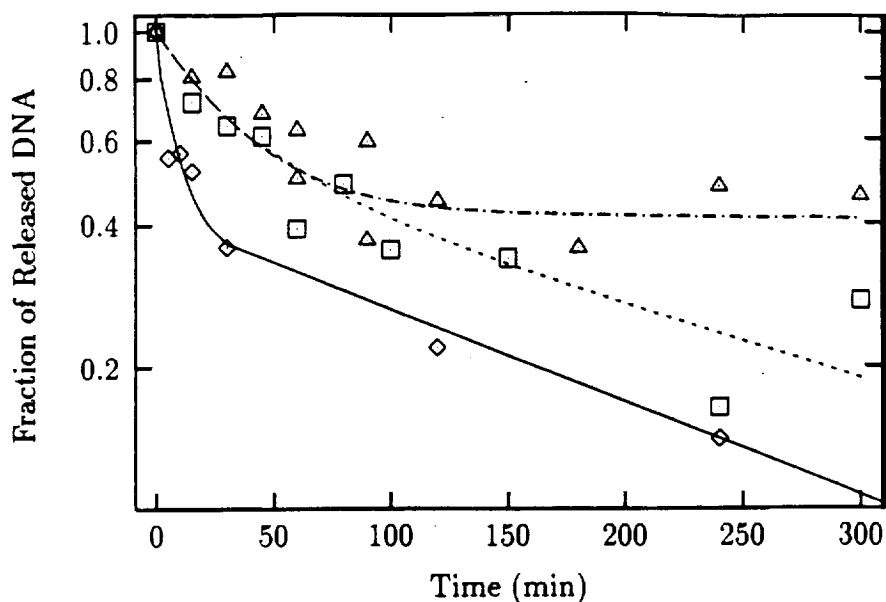


Figure 3: Rejoining of DNA double strand breaks in CHO-cells after irradiation with 250 kV X-rays (\diamond), 7.1 MeV/u argon-ions (\square) and 8.9 MeV/u gold-ions (\triangle). Initial damage in all three experiments is equivalent.

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