CELL CYCLE PHASE DEPENDENT EFFECT OF 3-AMINOBENZAMIDE ON DNA DOUBLE STRAND BREAK REJOINING IN X-IRRADIATED CHO AND xrs6 CELLS

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Although the contribution of poly(ADP-ribose) polymerase-1 (PARP-1) (EC 2.4.2.30) to DNA repair was implicated, there were numerous discrepancies in the experimental data and the controversies remain in spite of a considerable progress in our understanding of the repair processes. PARP-null mice and cell lines were shown to be hypersensitive to X/γ-rays but experiments with *in vitro* DNA repair systems did not indicate a direct participation of the enzyme in the break rejoining (reviewed by Jeggo [1], Sanderson and Lindahl [2]). As concerns DNA double strand break (DSB) repair, the effect of poly(ADP-ribose) polymerase inhibitors on DSB repair usually is difficult to demonstrate and, at best, transient. For instance, Rudat *et al.* [3] showed that PARP inhibition induced a shift from rapid to slow DSB rejoining.

It was previously reported that 3-aminobenzamide (AB) does not affect DSB rejoining when measured with the use of neutral comet assay in CHO-K1 (wild type) and xrs6 (radiosensitive mutant) cells [4]. Here, to evaluate DNA damage repair in the examined cells in different phases of the cell cycle, the results obtained for single cells in each experiment were grouped according to the distribution in the cell cycle. Cell population was divided into subpopulations corresponding to cell cycle phases on the basis of DNA content assessed from the total comet fluorescence.

Figure shows that the repair rate in CHO-K1 cells in all cell cycle phases is comparable. In AB-treated and irradiated cells, some delay in rejoining at a 15 min interval is seen in subpopulations in S and G2 cell cycle phases. However, the levels of residual damage are close in all subpopulations. Predictably, the (nonhomologous end-joining) NHEJ-defective xrs6 cells in G1 phase rejoin DSB more slowly than in S and G2 phases. This is in agreement with the known cell cycle specificity of NHEJ. AB does not impair the rejoining in G1 phase, but a delay in rejoining at a 15 min interval is seen in subpopulations in S and G2 cell cycle phases. The effect is similar to that observed in CHO-K1 cells. This result is consistent with the observations of homologous recombination dependence on PARP [5].

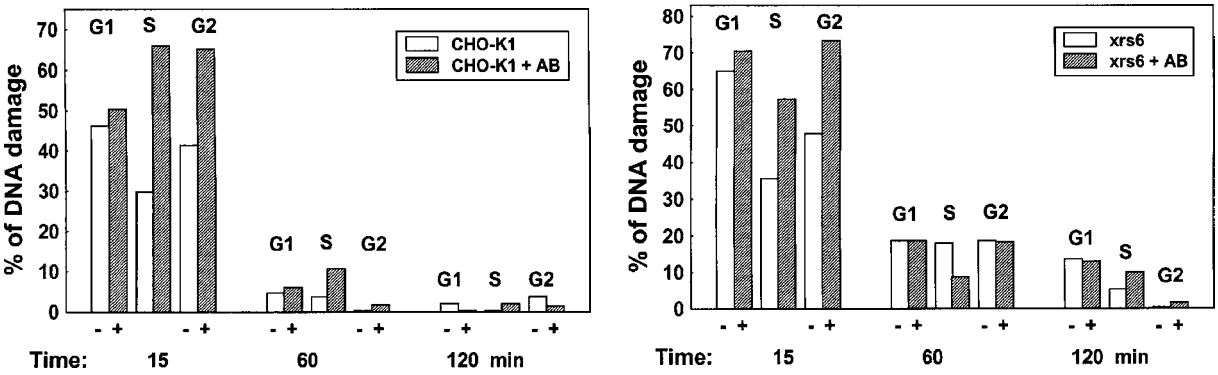

Fig. Cell cycle dependence of the course of DSB rejoining at 37°C in the presence or absence of 2 mM AB. Irradiation at time 0 with 10 Gy X-rays. Data for CHO and xrs6 cells from the neutral comet assay reported previously [4]. Cell populations were divided into subpopulations corresponding to cell cycle phases G1, S and G2, on the basis of DNA content assessed from the total comet fluorescence.

References

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FREQUENCY OF HOMOLOGOUS RECOMBINATION IN TWO CELL LINES DIFFERING IN DNA DOUBLE STRAND BREAK REPAIR ABILITY

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There are two major pathways for DNA double strand break (DSB) repair in mammalian cells: homologous recombination (HR) and nonhomologous end-joining (NHEJ). In certain situations, repair of DSB is restricted to either NHEJ or HR. The restriction in type of DSB repair raises the