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

Maria Wojewódzka

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].

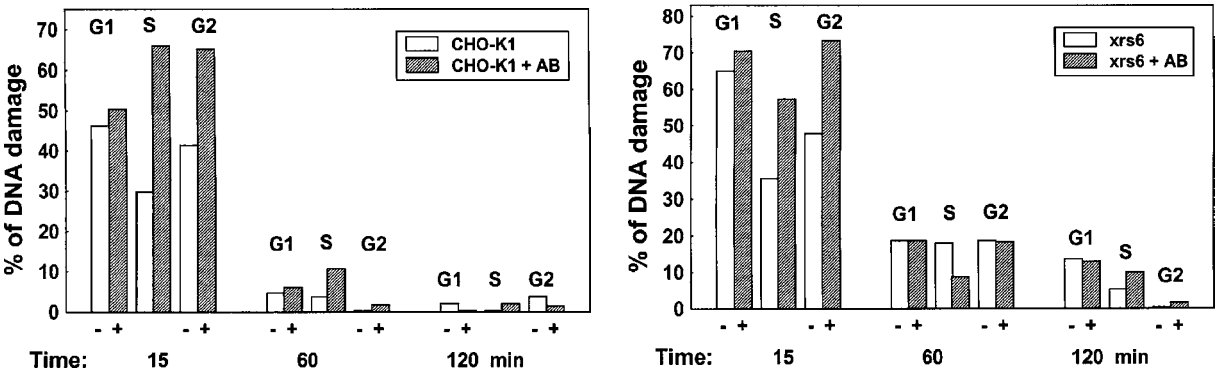

Fig. Cell cycle dependence of the course of DSB rejoining at 37°C in the presence or absence of 2 mM AB. Irradiation at time 0 with 10 Gy X-rays. Data for CHO and xrs6 cells from the neutral comet assay reported previously [4]. Cell populations were divided into subpopulations corresponding to cell cycle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

References

[1]. Jeggo P.A.: Curr. Biol., 8, R49-R51 (1998).
[2]. Lindahl T., Satoh M.S., Poirier G.G., Klungland A.: Trends Biochem. Sci., 20, 405-411 (1995).
[3]. Rudat V., Bachmann N., Kupper J.H., Weber K.J.: Int. J. Radiat. Biol., 77, 303-307 (2001).
[4]. Wojewódzka M.: In: INCT Annual Report 2002. Institute of Nuclear Chemistry and Technology, Warszawa 2003, p.110.
[5]. Chatterjee S., Berger S.J., Berger N.A.: Mol. Cell Biochem., 193, 23-30 (1999).

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

Maria Wojewódzka, Teresa Bartłomiejczyk, Marcin Kruszewski

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

question as to how pathway choice is regulated. To better understand the factors that modulate the choice of DSB repair pathway in mammalian cells, we investigated the frequency of spontaneous and X-ray-induced homologous recombination in NHEJ-competent (CHO-K1) and NHEJ-defective (xrs6) cells lines. To study homologous recombination in mammalian cells, we used pLrec plasmid that carries two non-functional copies of a bacterial gene, *lacZ* (β-galactosidase) in a tandem array [1]. The *lacZ* genes are divided by a selective marker gene, which provides resistance to the geneticin antibiotic – G418 (gene *neo*). Generation of a functional copy of the gene takes place in result of HR. So, β-galactosidase activity in the transfected clones was the measure of HR either spontaneous or X-ray-induced.

The transfected clones were selected on a selective medium containing 500 μg/ml G418. The clones with a single plasmid copy and with two copies of *lacZ* were identified by Southern blotting and PCR. Single copy transfectants were irradiated with 2 Gy X-rays and cultured in the medium ± G418. β-Galactosidase activity in cell extracts was measured 48 h after irradiation according to [2]. We found that the frequency of spontaneous (not shown) and X-ray-induced (Table) homologous recombination is enhanced in NHEJ mutant cells. In NHEJ competent cells lines Ku binding to both ends of DSB inhibits access by the homologous recombination machinery, so that when Ku is absent, HR is enhanced [3].

Table. β-Galactosidase activity (units per mg protein in cell extracts) in transfected cells as a measure of frequency of homologous recombination in CHO and xrs6 cells.

Clones of CHO-K1 cells transfected with a single copy of pLrec plasmid			
TK15	- G418	Control	1.31 ± 0.65 U/mg protein
		2 Gy	1.35 ± 0.71 U/mg protein
	+ G418	Control	0.75 ± 0.13 U/mg protein
		2 Gy	0.67 ± 0.10 U/mg protein
N11	- G418	Control	1.51 ± 0.39 U/mg protein
		2 Gy	1.66 ± 0.66 U/mg protein
	+ G418	Control	1.40 ± 0.30 U/mg protein
		2 Gy	1.41 ± 0.43 U/mg protein
Clones of xrs6 cells transfected with a single copy of pLrec plasmid			
S9	- G418	Control	2.47 ± 0.69 U/mg protein
		2 Gy	2.65 ± 0.20 U/mg protein
	+ G418	Control	2.04 ± 0.46 U/mg protein
		2 Gy	2.14 ± 0.50 U/mg protein
S15	- G418	Control	4.79 ± 0.67 U/mg protein
		2 Gy	5.02 ± 0.71 U/mg protein
	+ G418	Control	4.02 ± 0.43 U/mg protein
		2 Gy	4.85 ± 0.14 U/mg protein

References

[1]. Herzing L.B., Meyn M.S.: Gene, 137, 163-169 (1993).
[2]. Sanbrook J., Fritsch E.F., Maniatis T.: Molecular Cloning: A Laboratory Manual. Second edition. Cold Spring Harbor Laboratory Press, Cold Spring Harbor 1989.
[3]. Pierce A.J., Hu P., Han M., Ellis N., Jasin M.: Genes Dev., 15, 3237-3242 (2001).

EFFECTS OF SIGNALLING INHIBITORS ON SURVIVAL OF X-IRRADIATED HUMAN GLIOMA CELLS

Iwona Grądzka, Iwona Buraczewska

Growth factor receptor pathways are often activated in tumor cells by ionizing radiation or active oxygen species. Specific inhibitors have been developed that block the function of these molecules, thereby slowing cell growth and promoting cell death responses after radiation exposure [1, 2]. The study aims at investigation of the cellular response to combined treatment with X-rays and signalling inhibitor, with the use of human glioma cells. These cells are known for their high resistance to radio- and chemotherapy due to the high expression of the receptors for the epidermal growth factor (EGF) and insulin-like growth factor (IGF). We have chosen two related cell lines: M059 K and M059 J;