SIRTUIN INHIBITION INCREASES THE RATE OF DNA DOUBLE STRAND BREAK REPAIR IN xrs6 CELLS

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Histone deacetylases (HDAC) are an important member of a group of enzymes that modify chromatin conformation. Homologues of the yeast gene *SIR2* (silent information regulator) in mammalian cells code type III histone deacetylases (HDAC III, sirtuins), dependent on NAD^+ and inhibited by nicotinamide. It is assumed that in mammalian cells with damaged DNA, HDAC, including certain sirtuins, may modify chromatin structure and thus, alter the accessibility of the damaged sites for repair enzymes [1]. So far, however, there were no data directly confirming the effect of sirtuin inhibition on double strand break (DSB) repair processes in mammalian cells. We investigated the role of sirtuins in DSB repair using two Chinese hamster cell lines: wild type – CHO-K1 and radiation sensitive – DSB repair defective mutant line, xrs6. The latter is defective in DNA-dependent protein kinase (DNA-PK)-mediated nonhomologous end-joining (D-NHEJ) due to the deficiency in Ku80 protein. Here, we present the results of experiments with a specific sirtuin inhibitor – GPI 19015.

The cells were treated with sirtuin inhibitor 20 μM GPI 19015 at 37°C for 1 h and X-irradiated with 10 Gy without medium change. Using a recently validated neutral comet assay [2], we observed a relatively weak effect of GPI 19015 treatment on the repair kinetic in CHO-K1 cell line, as shown in Fig. In the DSB repair defective mutant cell line, xrs6, the increase in the rate of DSB repair was more pronounced although statistically significant only at the 15 min repair interval ($P=0.048$, $n=3$).

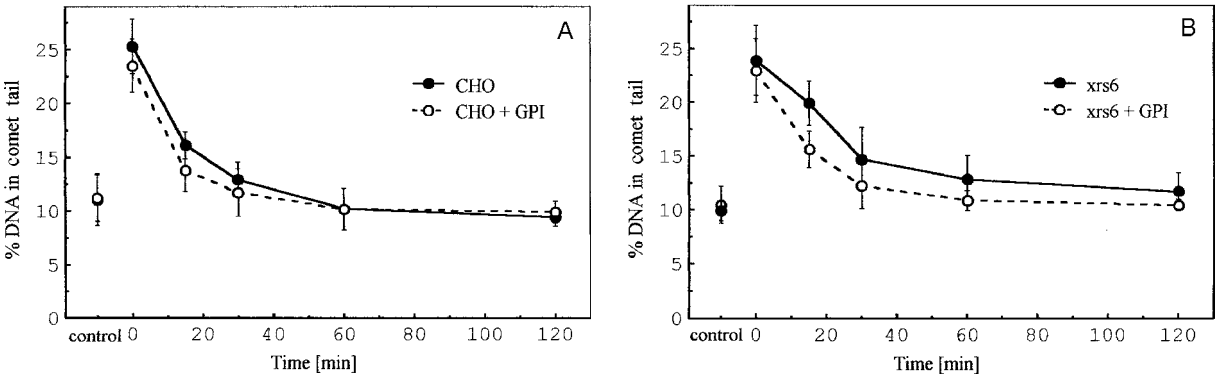

Fig. DSB repair in CHO-K1 (A) and xrs6 (B) cells, untreated or incubated with sirtuin inhibitor, 20 μM GPI 19015, at 37°C for 1 h and X-irradiated with 10 Gy without medium change.

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SHORT-TERM SIRTUIN INHIBITION DOES NOT AFFECT SURVIVAL OF CHO AND xrs6 CELLS

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In the preceding report, we described the effect of sirtuin inhibitor, GPI 19015 treatment on the repair of DNA double strand breaks (DSB) in CHO-K1 and xrs6 cells. In CHO-K1 cells, a relatively weak effect was noted at a 15 min repair interval. In contrast, in the DSB repair defective mutant cell line, xrs6, the increase in the rate of DSB repair was more pronounced. The cells were treated with sirtuin inhibitor 20 μM GPI 19015 at 37°C for 1 h and X-irradiated with 10 Gy without medium change. Applying the same experimental schedule, we determined survival. Here, the cells were cloned in fresh culture medium. This experimental schedule has been targeted on differentiation between effects on DSB repair and the late post-irradiation pro-

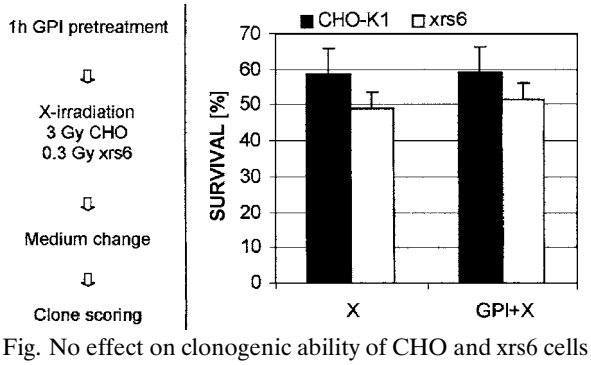

Fig. No effect on clonogenic ability of CHO and xrs6 cells treated with sirtuin inhibitor GPI 19015 20 μM at 37°C for 1 h, X-irradiated with 3 or 0.3 Gy (CHO and xrs6 cells, respectively) and cloned in fresh culture medium.

cesses such as pro-apoptotic signalling which is known to depend on sirtuins (reviews in [1,2]). We found that the short treatment with GPI 19015 before and during X-irradiation did not significantly alter survival, as shown in Fig. Prolongation of the treatment until clone scoring, however, did markedly enhance the lethal effect of irradiation (not shown).

The work was supported by the Polish Ministry of Scientific Research and Information Technology statutory grant for the INCT.

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BACKUP NONHOMOLOGOUS END-JOINING IS THE TARGET OF SIRTUIN INHIBITOR

Maria Wojewódzka, Marcin Kruszewski, Irena Szumiel

In the preceding reports, we described the effect of sirtuin inhibitor, GPI 19015 treatment on the repair of DNA double strand breaks (DSB) and survival in CHO-K1 and xrs6 cells. In CHO-K1 cells, a relatively weak effect was noted at a 15 min repair interval. In contrast, in the DSB repair (nonhomologous end-joining – NHEJ) defective mutant cell line, xrs6, the increase in the rate of DSB repair was more pronounced. The cells were treated with sirtuin inhibitor 200 μM GPI 19015 repair system) became more evident when we evaluated DSB rejoining 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. The results shown in Fig. show that at the 15 min repair interval in CHO-K1 the increase in rejoining was most marked in G1 and S phases. Predictably, untreated D-NHEJ-deficient xrs6 cells in G1 phase rejoined DSB much more slowly than the wild type CHO-K1 cells.

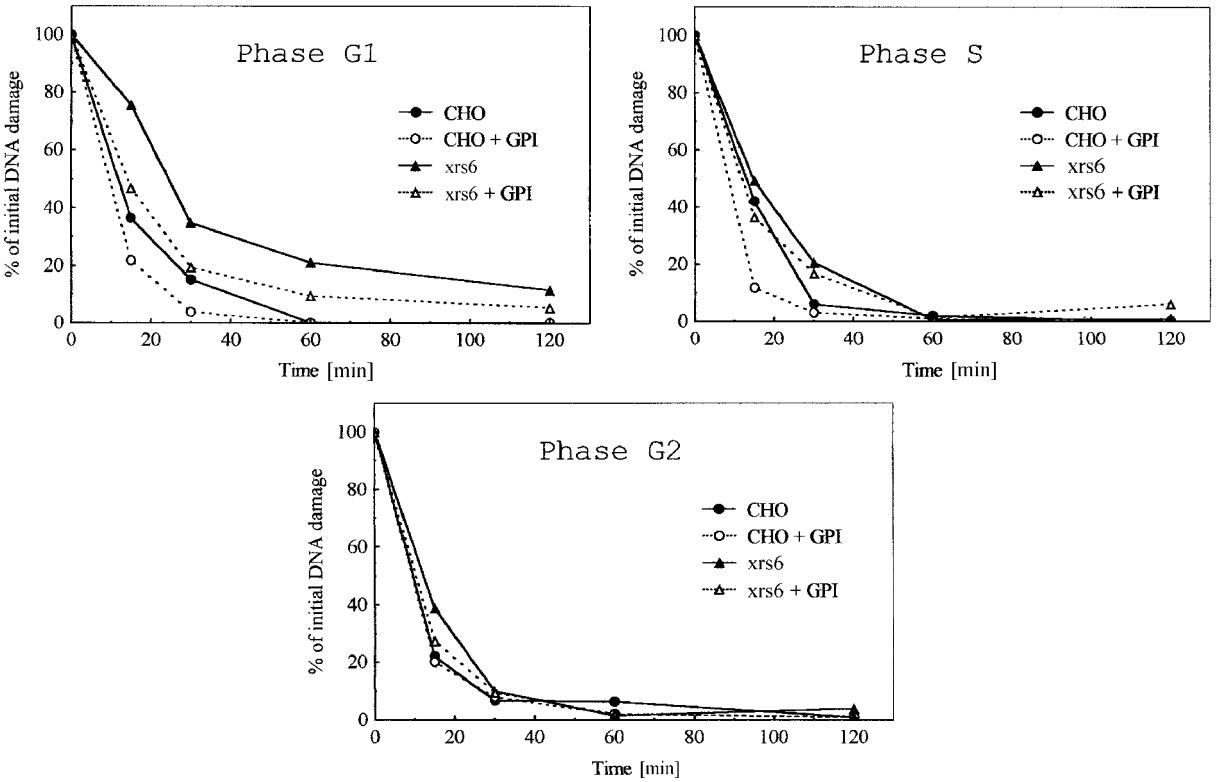

Fig. DSB repair in CHO-K1 and xrs6 cells, untreated or incubated with sirtuin inhibitor, 20 μM GPI 19015, at 37°C for 1 h and X-irradiated with 10 Gy without medium change. The results obtained for single cells in all experiments were pooled and grouped according to the distribution in the cell cycle.

at 37°C for 1 h and X-irradiated with 10 Gy without medium change. Applying the same experimental schedule, we determined survival and found that the short term treatment did not alter the clonogenic ability of both cell lines.

The difference between CHO-K1 (wild type) and xrs6 cells (deficient in DNA-dependent protein kinase – DNA-PK subunit Ku86 and hence, in dependent nonhomologous end-joining – D-NHEJ Nevertheless, sirtuin inhibitor accelerated the repair of DSB in G1 phase at early repair intervals, with the most pronounced effect at 15 min. In xrs6 cells in S and G2 phases the rejoining was improved at 15 min; at later intervals the difference between inhibitor-treated and untreated cells was lost. At these intervals, also the differences between wild type and mutant cells in S and G2 phases disappeared.