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).

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BACKUP NONHOMOLOGOUS END-JOINING IS THE TARGET OF SIRTUIN INHIBITOR

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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.

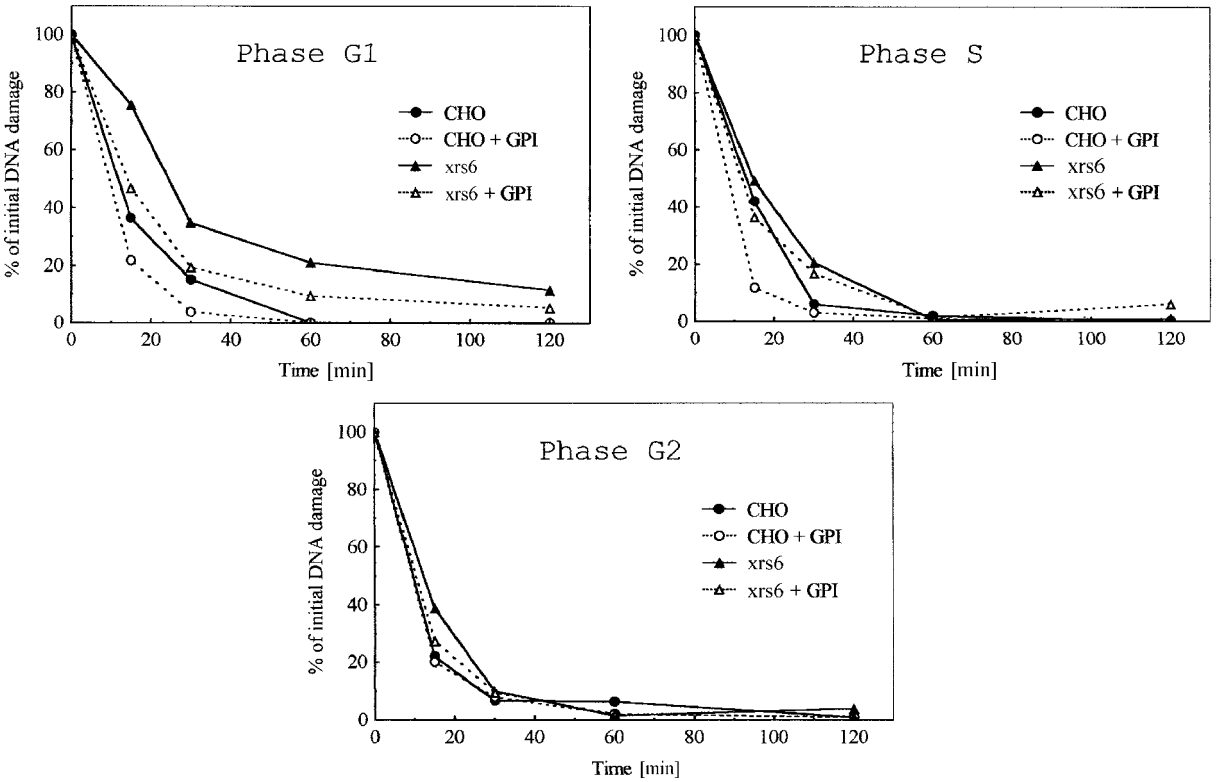

Fig. DSB repair in CHO-K1 and xrs6 cells, untreated or incubated with sirtuin inhibitor, 20 μM GPI 19015, at 37°C for 1 h and X-irradiated with 10 Gy without medium change. The results obtained for single cells in all experiments were pooled and grouped according to the distribution in the cell cycle.

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.

The possible reason of this effect may lay in the impaired DNA-PK D-NHEJ in xrs6 cells: DSB repair in these cells has to rely on the homologous recombination repair or DNA-PK independent (backup) B-NHEJ. The latter system is active in cells with impaired DNA-PK dependent rejoining system, D-NHEJ. It was reported by Wang and co-workers [1-3] that B-NHEJ is active in the G1 phase, supressed by DNA-PK, and involves ligase III instead of ligase IV. We conclude that the alternative (backup) route of DSB repair, B-NHEJ, is the DSB repair system affected by sirtuin inhibition to a greater extent than other DSB repair systems.

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