EFFECTS OF 1,4-DIHYDROPYRIDINE DERIVATIVE TREATMENT ON DOUBLE STRAND BREAK REJOINING AND SURVIVAL IN CHO AND xrs6 CELLS

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Derivatives of 1,4-dihydropyridine were described as efficient antimutagens and DNA repair modulators *in vivo* [1,2] and *in vitro* [3]. In particular, alkaline single cell gel electrophoresis (comet) assays showed that DHP (sodium 3,5-bis-ethoxycarbonyl-2,6-dimethyl-1,4-dihydropyridine-4-carboxylate, previously designated as AV-153 [3]) reduced the number of endogenously generated DNA strand breaks in untreated human lymphocytes and human promyelocytic leukemia HL-60 cells [3]. In HL-60 and Raji (human B-lymphoblastic leukemia) cells exposed to 2 Gy of gamma radiation or 100 μM hydrogen peroxide there was a statistically significant increase in the single strand break rejoining rate [3].

The purpose of the reported investigation has been to examine the effect of DHP on cell survival and repair of X-ray-induced DNA damage in Chinese hamster ovary (CHO-K1, repair competent) and xrs6 (repair deficient) cells differing in radiosensitivity and repair of double strand breaks (DSB). The present study is part of screening of 1,4-DHP derivatives synthesized in the Latvian Institute of Organic Synthesis.

Exponentially growing cells were incubated with various concentrations of DHP at 37°C for 1 h and X-irradiated. X-irradiation (ANDREX, Holger Andreassen, Denmark, 200 kVp, 5 mA) was at a dose rate of 1.2 Gy/min. Survival of CHO-K1 and xrs6 cells subjected to combined treatment (DHP+X) was determined by cloning. Cloning efficiency of both cell lines was about 50%. To determine the initial DNA damage, cells were irradiated with 10 Gy on ice. For the time course experiments, cells were irradiated at room temperature and incubated with the presence of DHP at 37°C for 15, 30, 60 and 120 min. DSB were estimated by the neutral comet assay in a recently validated version [4].

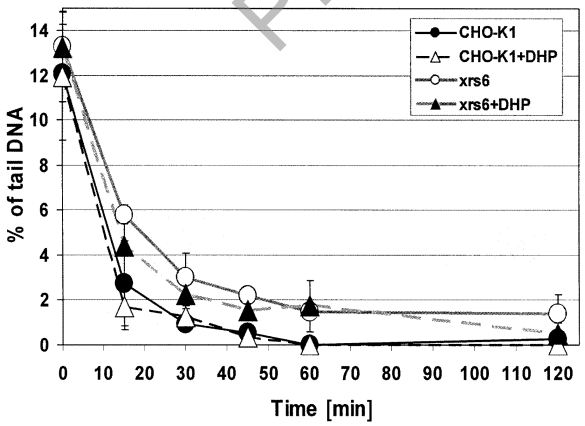

Fig.1. DSB repair estimated by the neutral comet assay in CHO-K1 and xrs6 cells incubated with 10 μM DHP at 37°C for 1 h and X-irradiated with 10 Gy without medium change. The value for control (unirradiated) cells was subtracted from that for irradiated cells. Four to five independent experiments were carried out for each treatment and percentages of DNA in the comet tail in 50 comets per experimental point were measured.

As shown in Fig.1, the difference in DSB rejoining between the DHP treated and untreated cells was very small not statistically significant. We observed only a weak effect of DHP treatment on the repair kinetics in xrs6 cells at the 15 min repair interval but the differences also are not statistically significant. In order to check the effect of the DHP treatment on survival, cloning experiments were carried out. The choice of dose for each cell line was such as to obtain a comparable survival level. As shown in Figs.2 and 3, there was no change in survival in result of the 1 h treatment with the

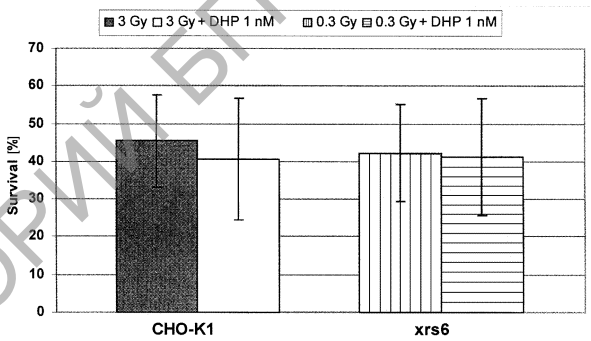

Fig.2. No effect of DHP on clonogenic ability of CHO and xrs6 cells treated with 1 nM DHP at 37°C for 1 h, X-irradiated and cloned in fresh culture medium supplemented with DHP; CHO-K1 cells, 3 Gy; xrs6 cells, 0.3 Gy.

DHP (1 nM or 1 μM) preceding X-irradiation. Higher concentration (10 μM) combined with higher radiation dose also had no effect (not shown).

The results presented above show that there is no effect of DHP treatment on survival and repair of X-ray-induced DNA damage in Chinese hamster ovary (CHO-K1) and xrs6 cells differing in

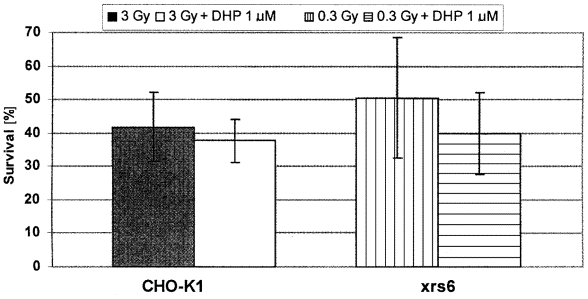

Fig.3. No effect of DHP on clonogenic ability of CHO and xrs6 cells treated with 1 μM DHP at 37°C for 1 h, X-irradiated and cloned in fresh culture medium supplemented with DHP; CHO-K1 cells, 3 Gy; xrs6 cells, 0.3 Gy.

radiosensitivity and repair of DSB. Since there are reports on various biological effects of this group of compounds when studied on whole organisms (*e.g.* chemical mutagenesis in *Drosophila mela-*

nogaster [1], micronuclei frequency in mice [2]) a possible explanation of the lack of effects *in vitro* is that the type of cell lines is not appropriate. Nevertheless, effect on DNA repair was claimed in [3] and the reason for these discrepancies, at present, is unknown.

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References

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