

DETECTION OF THE DNA DAMAGE AND ITS REPAIR

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ABSTRACT

The subject of this thesis is the observation of induction and reparation of DNA cross-links induced by *cis*-platin (*cis*-Pt). The modified Comet Assay was used to detection. The experiments were carried out on cell lines A-549, HeLa, HELF, XPA, XPC, CHO AA8 and CHO UV-20 (there are mutations in genes needed for keeping DNA unimpaired in cell lines XPA, XPC and CHO-UV 20).

The damage was induced by presence of 20, 40, 80 and 160 μM *cis*-Pt for one hour. The cells were divided into three groups for reparation observations at one, three and twenty three hour marks. Then the cells were incubated with styrene oxide (SO) for 30 minutes to enable the detection of cross-links. Alkaline Comet Assay was carried out afterwards, software LUCIA[®] Comet Assay was used to analyse the samples.

The cell lines A-549 and XPC showed the lowest induction of cross-links after one hour (87 and 86 % tail DNA), CHO-UV 20 showed the highest induction (70 % tail DNA). The number of cross-links rose after four hours in all types of used cell lines (the highest increase was observed in lines HELF and XPA, % tail DNA was reduced by 49 and 48 %). The reparation of DNA after 24 hours was registered in cell lines A-549 and CHO AA8 (44,68 % of cross-links was repaired in A-549 and 33,12 % in CHO AA8). The remaining cell lines showed an increased number of cross-links but no reparation.

As the last experiment, an MTT test was carried out to compare viability of the *cis*-Pt treated cells with the control group. The most sensitive cell line to the cytotoxic action of *cis*-Pt is cell line CHO UV-20 (decline of viability by 43,90 %), and the lowest level of sensitivity was in lines HeLa and CHO AA8 (decline of viability HeLa cells by 1,82 %; CHO AA8 by 9,86 %). Sensitivity of the other examined cell lines lies between these marginal values.