ABSTRACT

Nucleotide excision repair (NER) is one of the mechanisms of how to repair DNA damaged by the external influences. First of all, it plays the role when removing the results caused by UV radiation. The damaged bases in the form of pyrimidine dimers are enzymatically splitted out from DNA as part of oligonucleotides. The effect of the UV light on DNA is however manifested also indirectly, by oxidation of the bases. In our experiment we tried to find out whether the cells deficient in nucleotide excision repair are able to repair the damages induced only by oxidative agent.

We stated the extent of DNA damages in the cells in vitro and the ability of their reparation after treatment with the dilution of hydrogen peroxide in different concentrations. During the experiments we used the cell lines of a Chinese hamster, namely the parental cell line AA8 and their derivative UV-20, which is defective in gene ERCC1. This gene takes part in creation of nucleases participating in NER.

For measuring of DNA lesions we used the method of alkaline comet assay, the basis of which is alkaline single-cell gel electrophoresis. The principle of this method consists in the detection of DNA strand breaks originating in the damaged alkali-labile sites. We evaluated the number of DNA single-strand breaks. We detected either the breaks induced by hydrogen peroxide alone (SSB) or the breaks incurred by enzyme, which specifically recognises DNA sites, where hydrogen peroxide caused the oxidation of the base. The agent were enzyme endonuclease III splitting DNA in the sites of oxidized pyrimidine (endo III sites) and enzyme formamidopyrimidin-DNA-glykosylase splitting DNA in the sites of oxidized purine (FPG sites).

On the basis of our experiments we have found out that both the cells AA8, and the deficient cells UV-20 are able to repair oxidative damage. Both types of cells manifested comparable sensitivity in relation to hydrogene peroxide. Therefore, we have come to the conclusion that the defect in the mechanism of NER have no influence on the abilities of these cells to repair damages caused by the oxidative agent.