Abstract

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Title of doctoral thesis: Evaluation of DNA damage and repair in patients with non-small cell lung cancer due to drug therapy with platinum derivatives

Lung cancer is the most common worldwide cause of cancer-related death. This type of cancer is divided into two subtypes, the small cell and non-small cell lung cancer. Platinum-based chemotherapy is the mainstay of treatment of advanced stages non-small cell lung cancer, to that is this work focused on. The aim of this study was to investigate whether it is possible to use the comet assay method for monitoring the progress of treatment in these patients, and if it is possible to predict a patient's response to treatment or patient's survival.

Standard version or modifications of comet assay were used for the measurement of DNA damage or oxidative DNA damage and we also validated a modification of comet assay for measuring DNA crosslinks. This evaluation was carried out either *in vitro* in HeLa cells and in peripheral blood lymphocytes from healthy donors from Transfusion department of

University Hospital in Hradec Kralove and also in peripheral lymphocytes of patients with non-small cell lung cancer treated with platinum derivatives in the Pulmonary department in University Hospital in Hradec Kralove. Oxidative damage to DNA were also measured by ELISA where we evaluated the level of 8-OHdG in urine of patients.

At first we validated the comet assay method for the measurement of DNA crosslinks using a mutagen styrene oxide as a DNA breaking agent. The method was calibrated in *in vitro* conditions on HeLa cells and human lymphocytes from healthy donors. We have demonstrated that we are able to use this method to detect a cisplatin dose-dependent formation of DNA crosslinks. The results were subsequently verified in *in vivo* conditions in which we found that despite the considerable inter-individual differences, we are able to detect changes in induction and repair of DNA crosslinks in patients during chemotherapy. Further we evaluated DNA damage and repair by measuring single-strand breaks, DNA crosslinks and changes in the levels of DNA repair capacity (base and nucleotide excision repair) during chemotherapy. Then we focused on changes in levels of oxidative DNA damage during chemotherapy. We also compared input values of DNA damage and repair of patients and values of age-corresponding healthy controls. When comparing patients and controls, level of base excision repair was significantly higher in the control group, while the level of nucleotide excision repair in both groups was essentially the same. We found a significantly higher level of single-strand breaks immediately after administration of chemotherapy. Similarly, we found the highest incidence of DNA crosslinks immediately or one day after chemotherapy. We also found that the number of single-strand breaks increases throughout the chemotherapy (although nonsignificantly) and we can also find residual DNA crosslinks that persist in DNA even after the whole chemotherapy. We also compared the input values of repair capacity in patients who finished a whole chemotherapy and patients who died during it. We got similar results when comparing groups of patients and controls. However, the level of base excision repair was higher in the group of patients who finished a whole chemotherapy, while the level of nucleotide excision repair in both groups was essentially the same.

Data from our pilot study indicate that it is possible to use a modified comet assay for monitoring changes in the formation and repair of DNA crosslinks *in vitro* and in patients

during chemotherapy treatment. Furthermore, we confirmed the results of other studies dealing with DNA damage during the treatment of malignant diseases and the connection of the lack of base excision repair efficacy as a risk factor for these diseases. Moreover, our results suggest that although cisplatin is removed from the DNA by nucleotide excision repair, base excision repair plays also a very important role in the clinical condition of the patient and his survival.