

Abstract

In our study, we used the comet assay to assess the sort and range of DNA damage induced by sulfur mustard. Sulfur mustard belongs to chemical warfare agents and is embedded in the blistering agent category. It reacts with a wide range of macromolecules within cells (e.g. DNA, RNA and proteins) and induces for example alkylation of DNA.

The comet assay is a versatile and sensitive method for measuring single- and double-strand breaks in DNA, ultraviolet (UV)-induced pyrimidine dimers, oxidized bases, and alkylation damage.

The aim of the present study was to find out, how can be relaxed loops related to the amount of present strand breaks within DNA molecule. We were also interested in the mechanism of DNA damage caused by sulfur mustard.

The cell lines employed were HeLa, UV-20, A549 and AA8 cells. Initially, we assessed the influence between cross-links caused by sulfur mustard and strand breaks caused by styrene oxide. We found sulfur mustard to be highly potent to form cross-links even in low doses and to prevent relaxation of DNA loop caused by strand brakes.

Using selected enzymes, we have identified the sort of DNA damage induced by sulfur mustard. First we used endonuclease III, a protein from *E. coli* which acts both as N-glycosylase and a AP-lyase. It causes strand brakes in apurinic or oxidative damaged sites of DNA molecule. Step by step we compared the effects of endonuclease III and styrene oxide. We verified our findings with repair of cells influenced by sulfur mustard. The enzyme Alk A was applied in the same type of experiments. It is a DNA glycosylase, which catalyses the removal of 3-methyladenine and 7-methylguanine, as well as several other minor DNA lesions. Alk A is responsible for strand brakes in alkylating sites of DNA molecule.

Due to usage of UV-20 cells in tests with enzymes and sulfur mustard we could restrict the variety of DNA lesions, because of absence nucleotide excision repair in this cells. UV-20 cells probably use another types of DNA repair, e. g. base excision repair which is responsible for removing DNA-damaged bases, for example oxidized DNA bases or DNA alkylation. Lesions removed from DNA by the base excision repair include incorporated uracil, fragmented pyrimidines, *N*-alkylated purines and many others.

We found that high doses of sulfur mustard are responsible for another kind of DNA lesions, not only for cross-links. It could be oxidative or apurinic lesions, or another lesions, which are susceptible to the endonuclease III. And it could be alkylating damages, which are sensitive to Alk A. We think it could concern monoadducts with guanine.