SUMMARY

The aim of this study was to investigate the induction and repair mechanisms of DNA damage caused by sulphur mustard in specific cell lines and their relationship to cytotoxicity of sulphur mustard.

Sulphur mustard is a chemical warfare agent of the blistering agent category, which can be misused in local conflicts, terrorist attacks and during liquidation of its storage.

Sulphur mustard is an alkylating agent, which interacts with a wide range of cellular macromolecules including DNA, RNA and proteins. Sulphur mustard forms single – strand breaks, monofunctional guanine and adenine adducts, as well as interstrand cross-links involving the two guanines in interaction with DNA.

This study is aimed at cross-links. We used the Single Cell Gel Electrophoresis (SCGE, comet assay) for their detection. It is a method of evaluation of single cells ambeded in agarose. It is used for detection of DNA single strand breaks in a single cell.

In our modification of the method we determined cross-links, which make the alkaline DNA unwinding impossible. That is why we had to induce a standard number of single strand breaks in DNA by styreneoxide (at a certain concentration and exposure time) before the comet assay.

We studied DNA repair mechanisms of specific DNA lesions using specific cell lines with clearly determined deficits in certain phases of DNA reparation.

We used Chinese hamster ovary cells, line AA8, and their mutants UV—5 deficient in helicase activity and UV-20 deficient in endonuclease activity of DNA repair for our experiments.

There are several repair mechanisms possible for DNA damage: e.g. nucleotide excision repair (NER) as a response to action of many mutagenes, base excision repair (BER) which removes only one defective base and more complex recombinant repair.

In the experimental part of this study we also used several agents affecting particular stages of reparation processes. These were inhibitors of polymerisation phase of NER cytosinarabinoside (AraC) + hydroxyurea (HU) and specific inhibitor of poly(ADP-ribose)polymerase (PARP) 3--aminobenzamide (3-AB). We observed their affect on reparation processes.

In contrast with our hypothesis we found out that neither helicase repair activity, nor endonuclease repair activity of deficient cell lines play significant role in DNA reparation processes, but that necessary part of these processes is polymerisation of DNA. This is the reason why we came to a conclusin that DNA damage caused by sulphur mustard is repaired by recombination. But we did not prove the inhibition affect of 3-aminobenzamide on DNA reparation processes.

Results suggest that interstrand cross-links do not play an important role in the cytotoxicity of yperite. It seems that the main cytotoxic lesions are more likely monofunctional adducts of sulphur mustard with DNA adenines and guanines. This must be verified in subsequent studies.