Induction and repair of DNA cross-links induced by sulfur mustard in the A-549 cell line followed by a comet assay.

Abstract:
Sulfur mustard is a highly toxic chemical warfare agent with devastating impact on intoxicated tissues. DNA cross-links are probably the most toxic DNA lesions induced in the cell by sulfur mustard. The comet assay is a very sensitive method for measuring DNA damage. In the present study using the A-549 lung cell line, the comet assay protocol was optimized for indirect detection of DNA cross-links induced by sulfur mustard. The method is based on the additional treatment of the assayed cells containing cross-links with the chemical mutagen, styrene oxide. Alkali-labile adducts of styrene oxide cause DNA breaks leading to the formation of comets. A significant dose-dependent reduction of DNA migration of the comet's tail was found after exposing cells to sulfur mustard, indicative of the amount of sulfur mustard induced cross-links. The remarkable decrease of % tail DNA could be observed as early as 5min following exposure to sulfur mustard and the maximal effect was found after 30min, when DNA migration was reduced to the minimum. Sulfur mustard preincubated in culture medium without cells lost its ability to induce cross-links and had a half-life of about 15min. Pre-incubation longer than 30min does not lead to a significant increase in cross-links when applied to cells. However, the amount of cross-links is decreased during further incubation due to repair. The current modification of the comet assay protocol is expected to provide a sensitive and specific measurement of DNA damage induced by sulfur mustard.
assay provides a useful tool for detecting DNA cross-links induced by sulfur mustard and could be used for detection of other DNA cross-linking agents such as chemotherapeutic drugs.