Damage Responses Induced by the Sulfur Mustard Analog Mechlorethamine in Human HaCaT Keratinocytes

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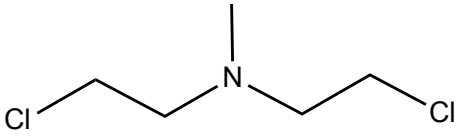
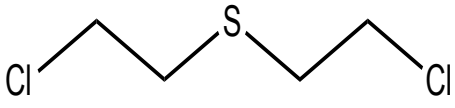
Mustard compounds, including sulfur mustard and nitrogen mustard, are vesicant agents which cause inflammation and blistering of skin upon exposure. These chemicals have been used in chemical warfare since World War I, and have also been used in conflicts like the Iran-Iraq war and recently by extremist groups like ISIS. In contrast, nitrogen mustards such as mechlorethamine (2-chloro-N-(2-chloroethyl)-N-methylethanamine; HN2) and chlorambucil are also used as chemotherapy drugs. In this study, the effects of mechlorethamine on the damage responses of HaCaT keratinocytes were assessed with the goal of elucidating the mechanism by which this compound is cytotoxic. We found that HN2 causes a time- and concentration-dependent inhibition of cell proliferation as measured by the 5-ethynyl-2'-deoxyuridine (EdU) assay. This is associated with cell cycle arrest in S and G2/M phases. Western blotting analysis revealed that HN2 treatment of HaCaT cells causes a time-dependent increase in the expression of p27 Kip1 and a decrease in the expression of CDK2. In addition, HN2 treatment increases protein autophosphorylation on H2AX (Ser139), p53 (ser15), HSP27 (Ser82), and protein acetylation on histone 4 (Lys16), indicating that HN2 activates DNA damage and stress response signaling pathways in HaCaT cells. This is supported by our findings that HN2 treatment caused an up-regulation of heme oxygenase-1 (HO-1). HN2 also alkylates p53 proteins forming several higher molecular weight cross-links suggesting that p53 is a direct molecular target for vesicant modification. This may lead to altering the function of p53 contributing to vesicant-induced toxicity. Taken together, our studies demonstrated that HN2 induces DNA damage and stress responses, and modulates cell cycle checkpoint signaling. These might be the critical mechanisms of vesicant-induced cell cycle arrest and cytotoxicity.



Nitrogen Mustard



Sulfur Mustard