Dose-Response Toxicity of Nitrogen Mustard on Mouse 3T3 Fibroblasts

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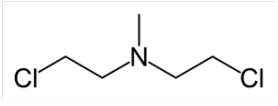
Vesicants including nitrogen mustard (NM) and sulfur mustard (SM) are bifunctional alkylating agents that induce severe fluid-filled blisters with prolonged wound repair and severe scarring. This is caused by disruption of the basement membrane components at the dermal-epidermal junction (DEJ). Fibroblasts have been shown to contribute to the deposition of basement membrane in the extracellular matrix (ECM). Upon injury, the expression and timing of provisional matrix components including osteonectin (secreted protein acidic and rich in cysteine, SPARC) in the ECM play regulatory roles for proper wound repair. The purpose of the present study was to investigate the cellular response to NM-induced toxicity on mouse fibroblasts [NIH/3T3 (ATCC® CRL1658TM)]. Cells were exposed to NM at low concentrations (1, and 3 uM) and high concentrations (10, and 30uM) for two hours. At 24 hours post-exposure, cell lysates were collected and proteins were extracted for western blots analyses. Expression of provisional matrix components (SPARC, Fibronectin) which are essential in the first stages of wound repair, matrix proteases (MMP9, ADAMTS4), and injury response markers (PARP, cleaved-Casp3 cyclophilin A, and PCNA) were examined. Our findings showed a dose-response toxicity to NM in markers for cell apoptosis and DNA repair. A marker of necrotic cell death (cyclophilin A) was also detected. Matrix proteases were significantly overexpressed at high doses of NM. However, there was a dose-response associated decrease, especially at 30uM, in components of the provisional matrix. The expression of SPARC corresponded to the dose-related reduced expression of proliferation marker (PCNA). The toxicity of NM at 30uM may prohibit the fibroblasts from proliferation, leading to the reduced expression of provisional matrix components for proper wound repair. Further research is necessary to find an underlying explanation for the dose-related decrease in wound repair markers.



NIH/3T3 mouse fibroblasts (ATCC® CRL1658TM)]
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2hr NM exposure
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Cell lysates 24hr post-exposure
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20ug total protein loaded per lane for WB