THE USE OF HUMAN EPIDERMAL KERATINOCYTES IN CULTURE AS A MODEL FOR STUDYING THE BIOCHEMICAL MECHANISMS OF SULFUR MUSTARD TOXICITY

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Human epidermal keratinocytes in culture were studied to evaluate their usefulness in demonstrating toxic events following exposure to sulfur mustard. Exposure of keratinocytes to sulfur mustard over a concentration range of 1–1000 μM HD, reduced NAD+ levels from 96% to 32% of control levels. When keratinocytes were exposed to a concentration of 300 μM HD, NAD+ levels began to fall at 1 hour and reached a plateau of 47% of control levels at 4 hours. Niacinamide, an inhibitor of the enzyme poly(ADP-ribose) polymerase, partially protected mustard-exposed cells against NAD+ depletion. It also protected cellular viability as assessed by vital staining 24 hours after exposure. This protection was not seen in long-term (72 hr) cultures. These studies suggest that human epidermal keratinocytes in culture can serve as a useful in vitro model for research into the biochemical mechanisms of sulfur mustard-induced cutaneous injury.

INTRODUCTION

Despite decades of medical research, the mechanism by which sulfur mustard (HD) induces skin vesication is not known (McAdams, 1956; Papirmeister et al., 1984; Renshaw, 1947; Schwarz, 1937) and no effective antidotes are available. Papirmeister et al. (1985) proposed a hypothesis, delineating the following events for the generation

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2. Key words: sulfur mustard, keratinocytes, vesication, in vitro models.
3. Abbreviations: HD—sulfur mustard, 2,2'-dichlorodiethyl sulfide, NAD+—nicotinamide adenine dinucleotide, ADP—adenosine diphosphate, HEK—human epidermal keratinocytes, PI—propidium iodide.
of HD-induced pathology: HD alkylates DNA, DNA strand breaks occur, poly(ADP-ribose) polymerase is activated and utilizes cellular NAD+ as substrate, NAD+ is depleted, glycolysis is inhibited, the hexose monophosphate shunt is activated, proteases are released, and pathology results. As predicted by this hypothesis, HD has been found to lower NAD+ levels in both human skin grafted to nude mice (Gross et al., 1985) and human leukocytes (Meier et al., 1987).

Human epidermal keratinocytes (HEK) in culture appear to be a suitable model for epidermal basal cells, the skin cells sensitive to the cytotoxic effects of HD. In this report, we demonstrate the use of HEK in determining concentration- and time-dependent reductions of NAD+ levels by HD, the cytotoxic dose response to HD, and in the therapeutic evaluations of niacinamide.

METHODS

Reagents and Cells: HD (96.8% pure) was obtained from the Chemical Research, Development and Engineering Center, Aberdeen Proving Ground, MD. All other reagents were purchased: niacinamide and propidium iodide (PI) (Sigma Chemical Co., St. Louis, MO), RPMI 1640, HEPES buffer, and gentamycin (GIBCO, Grand Island, NY). Normal Human Epidermal Keratinocytes (Clonetics, San Diego, CA) were purchased as second passage cells and maintained in Keratinocyte Growth Media (KGM, Clonetics), a modified MCDB 153 with 0.15 mM calcium for optimal growth (Boyce, 1983), in an incubator at 37°C and 5% CO2. Subcultures of HEK were obtained using Trypsin-EDTA and Trypsin Neutralizing Solution (Clonetics).

HD Exposure: When cells reached 60 to 80% confluence, they were trypsinized and plated at 100,000 cells/well in 24-well Falcon tissue culture plates (Becton Dickinson and Co., Lincoln Park, NJ) in a total volume of 2 ml. The next morning, KGM was removed and appropriate volumes of KGM containing HD were added. HD was added to the cells at concentrations ranging from 1 to 1000 μM. In the concentration-response experiments, NAD+ was acid extracted four hours later. In time-response experiments, NAD+ was acid extracted at selected time points from zero to seven hours after cells had been exposed to 300 μM HD.

Viability Studies: To determine HEK viability by propidium iodide (PI), the supernatant media containing the floating dead cells were collected in 12 × 75 mm tubes. Cells were trypsinized from the wells, pooled in respective tubes containing supernatant cells and trypsin neutralizing solution, and centrifuged at 250 × g for 10 minutes. The pellet was resuspended in 1 ml of KGM and PI (1 μg/ml) was added. After 3 minutes at room temperature, viability determinations were performed on an EPICS C flow cytometer operating with a 5 watt argon laser generating a 488 nm line at 200 mW (Coulter Electronics, Hialeah, FL).

Niacinamide Studies: Niacinamide was added to the plated cells, at a final concentration of 1 mM, immediately prior to the addition of HD. For the extended