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Apoptotic Cell Death: A Factor In Mustard Gas-Induced Dermal Pathology

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The weaponized chemical,sulfur mustard (HD), known historically as mustard gas, gained notoriety as the major chemical weapon of World War I. Its use resulted in over 300,000 battlefield casualties and changed the manner in which that war was raged. Since then, its use on several occasions by military combatants in smaller conflicts has resulted in an additional 100,000 military and civilian casualties. Regrettably, it remains today among the weaponized chemical agents of choice by some belligerent factions [1].

Whole body exposure to mustard gas causes immediate and delayed incapacitating injuries to exposed ocular tissues, skin and respiratory airways. Although investigative studies are suggesting that mechanisms of action of HD are associated with cellular biochemical consequences of DNA alkylations, the totality of the cytopathogenesis of HD toxicity remains investigatively elusive especially when addressing mechanisms of selected epidermal cell death in skin exposures.

Typically, HD-induced skin pathology in controlled animal studies is presented as occurring in sequential prevesication and vesication periods. During vesication, characteristic microvesicles progressively form at the skin basement membrane zone. Duringprevesication, selected epidermal basal cells are inevitably targeted for cell death [2]. The mechanisms by which HD causes cell death of selected basal cells remains obscure. Recent ultrastructural and immunohistochemical evidence suggests that induced apoptosis may be a factor [3].

The present study investigates further the role of induced apoptosis in epidermal basal cell degeneration caused by HD in hairless guinea pig skin. Anesthetized animals were exposed to 10µl neat HD by vapor cup for 8 min. Exposed skin sites were harvested from euthanatized animals by skin punch or by scalpel at selected postexposure times of 3 h, 5 h, 6 h, 12 h , 24 h. Sites were formalin fixed, processed in paraffin, sectioned and then stained with hematoxylin and eosin for routine histological evaluation. Replicate slide-mounted paraffin sections, either unstained or destained were immunoreacted for apoptosis according to an immunoperoxidase technical protocol for paraffin sections as described in an *in situ* oligo ligation kit (ISOL) (Intergen®, Purchase, NY) Unexposed skin sites, processed similarly, were used as controls.

Results confirm that apoptotic pathways are a cytotoxic mechanism of HD-induced epidermal basal cell death. Apoptotic markers were positive beginning at 5-6 h post exposure and remained distinctive up to 12 h (*Fig. 1*). Initially only a few cells were responsive. At later time periods, basal cells exhibiting apoptotic profiles increased significantly up to 12 h *(Fig. 2)* but were indistinquishable among the debris of cellular and tissue necrosis at 24 h postexposure. Comparative histopathological study of replicate slides stained with hemotoxylin and eosin, or replicate slides destained of hemotoxylin and eosin, established that not all basal cells presenting signs of HD-induced cytopathology, such as nuclear pyknosis, were positive for apoptotic markers.

This research was conducted in compliance with the Animal Welfare Act and other Federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, National Research Council, 1996. The facility where this research was conducted is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. The findings herein are the private views of the author(s) and are not to be construed as official or as reflecting the views of the U.S. Army or the Department of Defense.

References

[1] R. Zajtchuk, BG, Editor, *Textbook of Military Medicine*, Office of the Surgeon General, 1997.
[2] J. P. Petrali and S. Oglesby-Megee, *Microsc. Res. Tech.* 37 (3) (1997) 221-228.
[3] R.K. Kan et al., *Microsc. Microanal.* 7 (Suppl 2) (2001) 654-655.

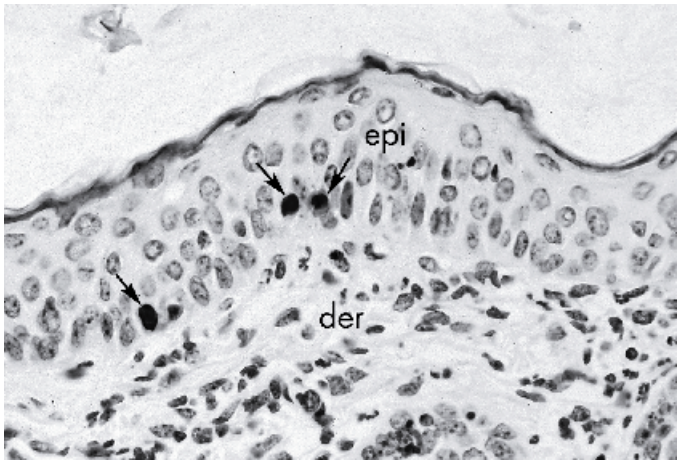

Fig.1. HD-exposed hairless guinea pig skin with epidermal basal cells specific for ISOL apoptotic markers (arrows). epidermis (epi); dermis (der).

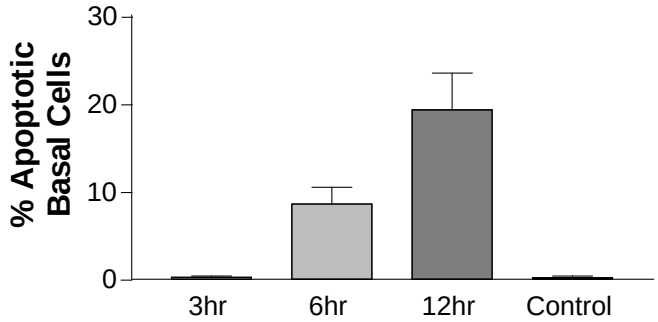

Fig.2. Percentage of ISOL positive epidermal basal cells at selected HD postexposure times.

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