

Electron Microscopical Study on Skin Lesions Induced by Sulphur Mustard

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ABSTRACT

Mustard gas or sulphur mustard (SM) was applied on the dorso-caudal surface of mice to examine the effect of SM on the collagenous extracellular matrix (ECM) of the mouse skin. The animals were sacrificed on first, third and sixth days to study structural changes in the ECM of the mouse skin. Disruption of ordered arrangement of collagen bundles was observed on different days. The bundles of collagen fibres were found opened up into small fragments on the third day post-exposure. Infiltration of polymorphonuclear cells and damage to the wall surrounding the blood vessels were observed. Reformation of ordered layers of collagen bundles and the narrowing of voids were taken as indicators of recovery which became perceptible on the sixth day after exposure.

1. INTRODUCTION

Bis (2-chloroethyl) sulphide, commonly known as mustard gas or sulphur mustard (SM), is a highly reactive alkylating agent and forms blisters in human skin¹. The blisters are painful and slow to heal². It is reported that the pathology of SM-exposed human skin bears resemblance to that of animal skin to a certain extent³. Subsequently many novel in vitro models have been developed to study aspects of SM behaviour and toxicity on animal skin¹⁻⁴. Cellular injury caused by SM and recorded by electron microscopy showed condensation of heterochromatin, blebbing of the nuclear membrane and formation of large perinuclear and cytoplasmic vacuoles^{5,6}. Although the degenerative changes are noticed early in cells, it is expected that the extracellular matrix (ECM) which constitutes the major component of the skin will also be affected. Though the ECM has been shown to be critical for nonlymphoid cells in regulating a diversity of cell functions⁷, the importance of SM interaction with ECM has been largely ignored and only a few attempts have been made to study the effect of SM on the extracellular matrix⁸. Recent studies have revealed that the ECM plays an important role in remodelling of injured skin

tissues, and during wound healing, cell and matrix interact closely and iteratively^{9,10}.

The present study was undertaken to examine the effect of SM on the collagenous ECM of the mouse skin to follow the course of injury as well as the onset of recovery. The morphological changes were studied utilising a scanning electron microscope.

2. MATERIALS AND METHODS

Swiss Albino male mice weighing 25 ± 3 g were kept in polypropylene cages. The animals had free access to food and water. Sulphur mustard (98 per cent pure) synthesised in the Chemistry Division of the Defence Research & Development Establishment was applied on the clipped area on the dorso-caudal surface of the animals. A single dose of SM (LD_{50}) (154.7 mg/kg percutaneous) was applied on the area of approximately 1 cm^2 . The animals were sacrificed on first, third and sixth days after the treatment. In each group, six animals were taken. Skin tissues were processed for electron as well as light microscopy at 4°C for 12 hr using formaldehyde-glutaraldehyde fixative¹¹. Tissues were post-fixed at 20°C for 2 hr with 1 per cent osmium tetroxide solution. Paraffin wax

blocks, prepared for the study, were used to prepare 10-12 micron thick sections using a rotary microtome. Dewaxed sections were coated with a thin layer of gold in JEOL JFC-1100 sputter coating unit and examined at 5 kV in a JEOL JSM-840 scanning electron microscope (SEM). Simultaneously 5 micron thick sections were cut from the paraffin wax blocks and examined in a light microscope following staining with hematoxylin and eosin.

3. RESULTS AND DISCUSSION

Control skin from the unexposed portion of the animal showed a distinct cornified epidermis (arrow) as well as a well-aligned and compact collagenous dermal matrix (Fig. 1a). Figure 1b shows intact lipocytes boundaries in the control specimen. In preparing the



Figure 1a. Control skin showing cornified epidermis (arrow) as well as compact matrix. Embedded hair follicle roots are also seen (arrow head) (500×).

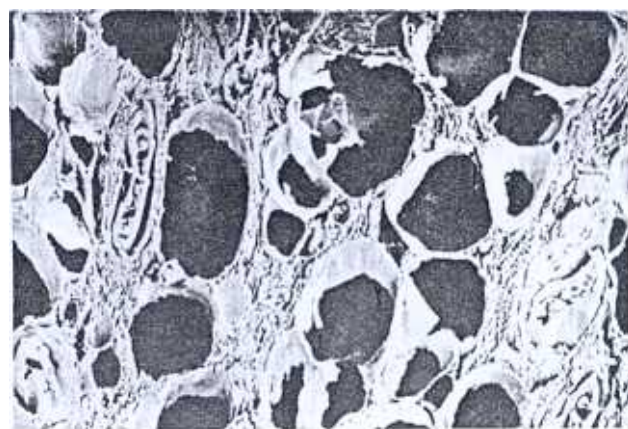


Figure 1b. Control skin showing intact lipocyte cell boundary surrounded by the collagenous support (500×).

skin tissues for electron microscopy, lipids were lost from lipocytes and were seen as a delicate network of structure. The lipocytes are surrounded with collagenous support from all the sides. The 24 hr treated skin showed loosening of collagenous matrix resulting in the distortion of the matrix. The bundles of collagen fibre were opened up (Fig. 2a). Figure 2b shows bundles of collagen fibre after the treatment.

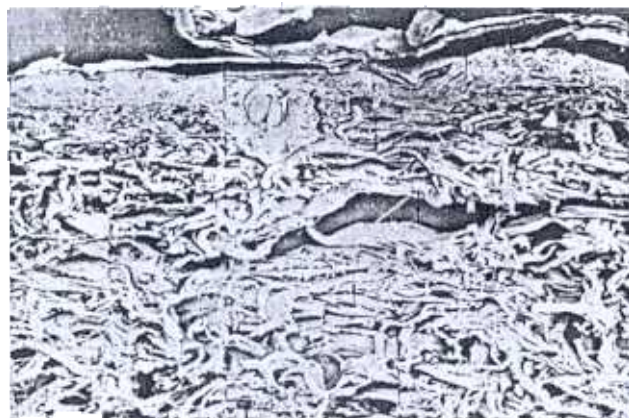


Figure 2a. Morphological changes after 24 hr; Loosening of collagenous matrix resulting in void formation (500×).



Figure 2b. Well separated bundles of collagen fibres but still maintaining cable like structure (2000×).

The extent of damage to collagenous ECM was extensive on the third day post-exposure. The collagenous fibrils were opened up from the well compact configuration. Large gaps in the ECM were noticed. At places, many of these fibres were found ruptured and their length shortened. The underdeveloped fibres and their roots were dislodged due to the loosening of surrounding collagenous support

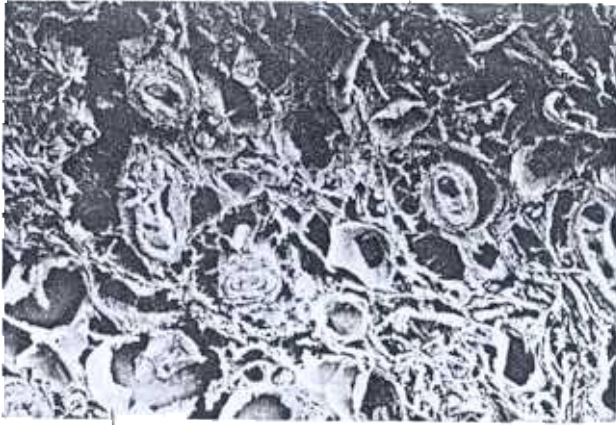


Figure 3a. Morphological changes at three days post-exposure. Distortion of ECM and fibrillation of collagen matrix (500 \times).

(Fig. 3a). As compared to the 24 hr treated skin, where the collagen fibres constituting the matrix retained their cable like structure, a three-day old skin showed extensive fibrillation and disalignment of collagen fibres after exposure to SM (Fig. 3b). The light microscopy observations revealed extensive edema, infiltration of polymorphonuclear (PMN) cells and dilation of blood



Figure 3b. Extensive fibrillation resulting in the fragmentation of collagenous bundle on the third day post-exposure, (2000 \times).

vessels. The collagenous wall supporting the blood vessels was also damaged at places (Fig. 3c). Damage to the collagenous support of blood vessels was confirmed (Fig. 3d) by transmission electron microscopy (TEM). The observed changes on the sixth day post-exposure showed a reverse tendency in that the enlarged intercellular gaps in the dermal region underwent a noticeable reduction (Fig. 4a). Overall improvement was observed in the alignment of

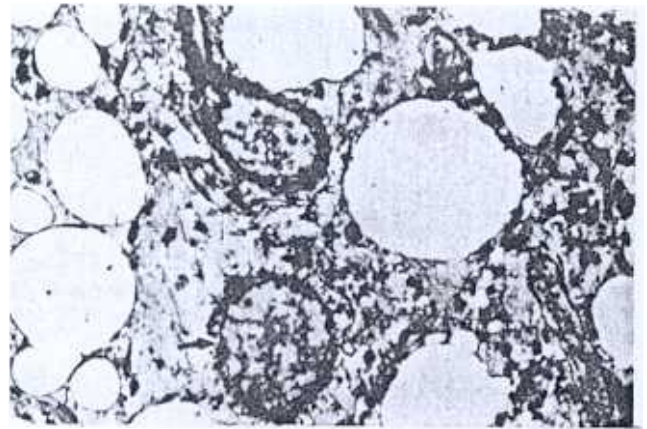


Figure 3c. Light micrograph showing infiltration of PMN cells and damage to collagenous wall of the blood vessel (arrow) (500 \times).

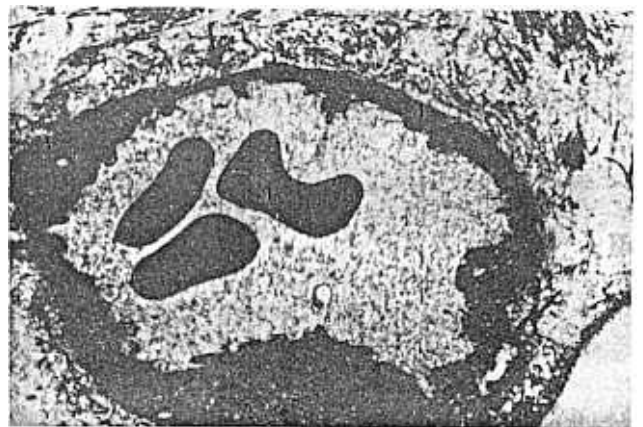


Figure 3d. TEM of blood vessel, sign of rupture of collagen wall supporting the blood vessel (4000 \times).



Figure 4a. Restoration of order in dermal as well as epidermal regions. Fibre bundles are aligned parallel to epidermis on the sixth day post-exposure (1000 \times).

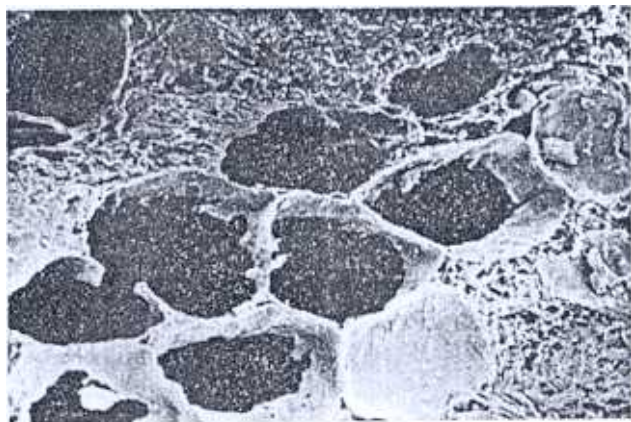


Figure 4b. Restoration of lipocyte boundaries (1000 \times).

collagenous matrix both in the dermis and in the epidermis resulting in the restoration of order. Likewise the lipocyte boundaries were also restored to an extent at many places. Figure 4b shows restoration of lipocyte boundaries.

The local effects of SM are reported to cause destruction of target organ, accompanied by vascular alteration, tissue swelling and generalised inflammation^{1,5-6}. Measurements done in an earlier study on changes in skin thickness after exposure to SM indicated the extent of tissue swelling in mice⁸. The degradation of cellular and extracellular constituents that accompanies cell necrosis is likely to increase the colloidal osmotic pressure gradient across the vascular wall which may account for the massive fluid accumulation in SM-induced edema^{12,13}. Excessive inflammation which results in the retention of edematous fluid may cause more damage than that attributable to direct SM toxicity¹. It is well known that SM is an alkylating agent with lipophilic properties and due to the electrophilic nature of the sulphonium ion it may not only react with (cross-link) DNA but also with other biomolecules¹⁴.

The combined effect of these two factors, namely, the accumulation of edematous fluid (generating physical forces) and the action of SM with those biomolecules (lipid being one of the structure substances) which bind the collagenous bundles, could lead to the loosening of collagenous support of the dermis which, in turn may lead to various histological changes (Figs 1b to 3b). Recent studies done on guinea pig skin revealed that collagen fibres lose their characteristic periodicity after SM exposure (communicated by authors). In vitro experiments on

biologically active analogues of the ECM indicated that the filling up of the ECM plays a vital role in the remodelling of damaged skin. Studies on the effect of SM in organ culture from rabbit skin indicated that the healing occurred under the crust by migration of epidermal cells from the undamaged skin as well as from hair follicles cells¹⁵.

This study indicates that SM induced the disruption of the extracellular matrix and involved collagenous fibres. The disruption of the ECM could be due not only to the forces generated during the course of retention of edematous fluid but also to the action of SM on the collagenous component of the ECM. Since all alkylation products exhibit great stability under physiological conditions, it is likely that the cross-linked collagenous ECM will take a long time to return to normal structure and subsequently for complete recovery which is the case with SM toxicity in animals and human skins. The SEM observations of the ECM presented in this report lend support to earlier studies in spite of different animal models used^{1,5,15}.

ACKNOWLEDGEMENTS

The authors thank Dr. R.V. Swamy, Director, Defence Research & Development Establishment for the encouragement during the course of the present study. Thanks are due to Dr. R.C. Malhotra for the synthesis of the compound used in this work and Dr. P.R. Malhotra for providing the experimental animals. The help given by Mr. G.R. Khanvilkar for typesetting is thankfully acknowledged.

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