**PROTEASE INHIBITORS PREVENT MICROVESICATION IN SULFUR MUSTARD WOUNDS IN HUMAN SKIN EXPLANTS**

Bernd Liesenfeld1, Marijke Moé2, Gregory Schultz2,3, Quick-Med Technologies, 2 TNO Laboratories, Netherlands, 3University of Florida, *corresponding author

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**Summary**

Sulfur Mustard is a vesicant (blistering) agent that was widely used in WWII, and now was recently used in Iraq by Saddam Hussein. Because the agent is relatively easy to manufacture and weaponize, US Intelligence estimates consider it as a significant terrorist threat. The mechanisms by which sulfur mustard elicits injury on skin is thought to involve proteases (and likely, other inflammatory agents), which illustrates significant similarity in the biochemistry of the damage mechanisms with some types of chemical and thermal burns.

**Exposure of skin to sulfur mustard (also often called HD or SMG) is thought to disturb the balance between basement membrane protein synthesis, by keratinocytes, and their degradation by proteases. This disturbance causes a loss of adherence between epidermal cells and basement membrane collagen (type IV) and can lead to the formation of microvesicles (vesication), a process that is considered a reliable indicator of wound severity.**

**Materials and Methods**

The test materials, ilomastat, doxycycline and α1-PI were supplied by Quick-Med Technologies. BB94 was kindly provided by Brithe Bech, Odense, Unk. Karacteristisk basale medium (BGM) C1005 was obtained from BovineHeart, Van Nuys California. HD was supplied by the Biocombinatory Chemical Branch of Two Prime Mammal Laboratory and has a purity of >97%. Human mammary skin was obtained from cosmetic surgery with informed consent of the patient. Human mammary skin was exposed to saturated HD vapor at 25°C for five minutes using a vapor cup device (Mo et al., 1999). HD vapor exposed skin pieces of 0.25 cm² were floated on the dermal side down in KBM supplemented with CaCl₂, at a final concentration of 1.4 mg/ml (1 ml medium of a 12 well culture plate). The medium was replaced every 24 hours. The skin explants were kept at 37°C, in an atmosphere of 6% CO₂ in air or 4% CO₂ (Van et al., 1999).

**Results**

Loss of the attachment of epidermal cells to the basement membrane is postulated to be a specific cause of sulfur mustard (HD)-induced vesication of the skin. Excessive proteolytic activity has been suggested to play a major role in this vesication due to an altered balance between production and degradation of ECM proteins and cell membrane proteins. Since matrix metalloproteinases (MMPs) and serine proteases (elastase) are probably involved in degradation of basement membrane proteases, two inhibitors of MMPs and one inhibitor of serine proteases were tested on their effectiveness in preventing epidermal dermal separation. The compounds were tested in an in vitro human skin model, consisting of skin pieces that were exposed to HD in organ culture for 48 hours. Histological evaluation of the cultured skin explants showed microvesication and extensive epidermal necrosis in skin that was not treated with a protease inhibitor. The presence in the culture medium of 1% MilliQ water (Figure 4A), 1% doxycycline (Figure 4B), and 1% α1-PI with 2.5 mg/ml (Figure 4C) showed an appearance in the epidermal layer different from normal. A separation of the intercellular space between the epidermal layers was observed. The simultaneous presence in the culture medium of α1-PI with doxycycline augmented this phenomenon, whereas at α1-PI in combination with ilomastat did not alter the histo logical appearance of the skin.

**Conclusions**

α1-PI was shown to be effective at preventing microvesication in the human skin explant model. Low concentrations of ilomastat (50 µg/ml) and 2 mg/ml α1-PI administered immediately after HD exposure of the skin.

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**Table 1** (right): Summary of findings of the study.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Effect on HD-induced microvesication.</th>
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<tbody>
<tr>
<td>No HD</td>
<td>No microvesication detected</td>
</tr>
<tr>
<td>HD + α1-PI</td>
<td>No microvesication detected</td>
</tr>
<tr>
<td>HD + doxycycline</td>
<td>Complete protection</td>
</tr>
</tbody>
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**Figure 1:** Vascular injury after sulfur mustard exposure. Exposure of human skin explants to saturated HD vapor at 25°C for five minutes using a vapor cup device (Mo et al., 1999). HD vapor exposed skin pieces of 0.25 cm² were floated on the dermal side down in KBM supplemented with CaCl₂, at a final concentration of 1.4 mg/ml (1 ml medium of a 12 well culture plate). The medium was replaced every 24 hours. The skin explants were kept at 37°C, in an atmosphere of 6% CO₂ in air or 4% CO₂ (Van et al., 1999).

**Figure 2:** A and B: Effects of Sulfur Mustard on human skin explants. A is a typical appearance of the skin with severe necrosis and microvesication, and B is a sulfur mustard-exposed skin explant showing moderate cellular condensation, as well as microvesication (pass indicated by red arrows) at BM.

**Figure 3:** Images C through F: Effects of treatment with the agents on HD-exposed skin explants. Doxycycline (J) and ilomastat (C) were added to the culture medium at concentrations of 46 µg/ml, but did not induce microvesication at 46 µg/ml. A-1 PI also did not provide any protection but did indicate damage to BM at concentrations >100 µg/ml. The other agents at use concentrations did not cause any skin damage.

**Figure 4:** Images J, L, N (below) Effects of treatment with the agents on HD-exposed skin explants. Doxycycline (J) and ilomastat (C) were added to the culture medium at concentrations of 46 µg/ml, but did not induce microvesication at 46 µg/ml. A-1 PI also did not provide any protection but did indicate damage to BM at concentrations >100 µg/ml. The other agents at use concentrations did not cause any skin damage.

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References:

2. Roy Carr - Director of Business Development, Medical Devices, Email: rcarr@quickmedtech.com (561) 771-1304
3. Gerald Olderman - Ph.D., VP, Research & Development, Email: goodwill@quickmedtech.com (561) 771-1304

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