Chronic senescence in primary human dermal fibroblasts due to single dose exposure to sulfur mustard

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Introduction

Erythema, skin blisters and chronic wounds are the result of dermal exposure to sulfur mustard (SM) (Fig. 1) or ionizing radiation [1, 2]. Similar to ionizing radiation, SM interacts with DNA leading to cross-links and consequently to mutation or cell death. Cutaneous wound healing is dependent on different cell types including fibroblasts which contribute to granulation tissue formation [3]. It is known that different cell types react to exposure of SM or ionizing radiation with transition to a senescent state which might have a negative impact on wound healing [4]. In this study, we investigated the SM sensitivity and the induction of chronic senescence in primary human dermal fibroblasts (HDF).

Methods

HDF were exposed to SM at final concentrations from 0.03 µM to 1000 µM or solvent control for 24 h and the XTT assay was used to determine the 50 % lethal concentration (LC50). H2O2 was used as positive control for senescence induction [4]. HDF survival after exposure to 300 µM - 2000 µM H2O2 for 24 h was determined by the XTT assay. Sub-lethal concentrations were used to investigate the induction of senescence in HDF by SM and H2O2. Cells were exposed to 3 – 65 µM SM or 500 µM H2O2 and senescence-associated β-galactosidase (SA-β-gal) was stained histochemically over 31 days.

Results

Table 1: LC50 values determined by the XTT assay. Data are represented as mean ± SD.

<table>
<thead>
<tr>
<th>SM</th>
<th>LC50 (µM) ± SD</th>
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<tr>
<td>3.1 ± 0.7</td>
<td>439.6 ± 12.4</td>
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<tr>
<td>12.7 ± 2.0</td>
<td>492.6 ± 9.6</td>
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<tr>
<td>29.2 ± 3.0</td>
<td>522.6 ± 8.0</td>
</tr>
<tr>
<td>62.6 ± 5.0</td>
<td>565.8 ± 5.7</td>
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<tr>
<td>161.7 ± 7.3</td>
<td>612.5 ± 4.1</td>
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Fig. 3: Percentage of SA-β-gal positive cells counted over 31 days. Three randomly selected picture sections per group (see Fig. 5) from three independent experiments were analyzed. Data are represented as means with 99 % confidence intervals (coloured ribbons).

Fig. 5: Concentration- and time-dependent senescence induction Images of SA-β-gal staining (blue) after single dose exposure to solvent control, SM and H2O2 at day 0. Cells were counterstained with nuclear fast red (red). Stable senescence was induced by 24, 40 and 65 µM SM. Senescence induction after exposure to 3 and 13 µM SM was not persistent. 500 µM H2O2, used as positive control for senescence induction, was insufficient for stable induction. Senescent cells showed an increased cell size. Scale bar, 200 µM.

Summary

HDF exposure to sub-lethal SM concentrations results in chronic senescence. HDF dysfuction may contribute to the chronic cutaneous wound healing disease after SM exposure. More research concerning the secretome might give an insight into altered cytokine release of senescent HDF. It is further necessary to investigate ionizing radiation as trigger of a senescent state in HDF. This novel pathomechanism provides a possible new therapeutic target to improve wound healing after exposure to SM or ionizing radiation.

References: