

Effects of hypoxia on primary keratinocytes and fibroblasts: Influence of sulfur mustard

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Sulfur mustard (SM) is a chemical warfare agent that provokes severe inflammation and blistering upon exposure to the skin accompanied by disturbed wound healing. The potential use of SM in terroristic assaults amplified the interest in understanding the underlying cellular and molecular pathomechanisms in order to improve therapeutical intervention.

Similar to chronic wounds, injuries induced by exposure of the skin to SM are associated with a very low level of oxygen (=hypoxia) in the tissue. Thus, we hypothesized that hypoxia may contribute to the pathophysiology of delayed wound healing observed in the skin upon exposure to SM.

In our studies, we used primary normal human epidermal keratinocytes (NHEK) and primary normal human dermal fibroblasts (NHDF). These cells represent the major cellular components in epidermis and dermis and substantially promote the repair and regeneration of injured skin tissue. Under hypoxic conditions, the hypoxia-inducible factor 1 α (HIF1- α) signaling pathway is known to play a key role in the control of important cellular processes including proliferation and migration. Therefore, NHEK and NHDF were intoxicated with SM at several concentrations (30 μ M, 60 μ M and 100 μ M) and subsequently cultivated under hypoxic (1% O₂) and normoxic (21% O₂) conditions for different time intervals.

The results of our studies demonstrated a significant upregulation of HIF1- α and its target genes Bcl-2 and nineteen-kilodalton interacting protein-3 (BNIP-3), vasoactive endothelial growth factor(VEGF) and matrix metalloproteinase-2 (MMP-2) under hypoxic conditions compared to normoxia as determined by qRT-PCR, Western blotting and/or zymography. Interestingly, secretion of MMP-9 was strongly increased in NHEK after 6 days of exposure to hypoxia. Moreover, hypoxia led to a significant reduction in proliferation (BrdU Assay) and non-directed as well as directed invasion of NHEK and NHDF (Transwell assay). Preliminary data on the influence of SM on HIF1- α signaling in NHEK and NHDF indicate a decrease in HIF1- α biosynthesis under hypoxic conditions upon treatment of the cells with SM. These findings suggest that SM may interfere with the hypoxia-induced accumulation of HIF1- α which represents an important step in early stages of normal wound healing and tissue repair.

In summary, our findings show that the HIF1- α signalling is induced in primary keratinocytes and fibroblasts under hypoxic conditions. SM may cause a dysregulation of the HIF1- α signalling pathway in skin cells that possibly results in disturbed wound healing.

Biography

Janina Deppe was born in Munich in 1986. She studied biology at the Ludwig-Maximilians-University (LMU) of Munich with a focus on cell biology, pharmacology/toxicology and human biology. In December 2011 she started her PhD at the former Department of Clinical Chemistry and Clinical Biochemistry and now referred to as Institute for Cardiovascular Prevention of the Medical Faculty of the LMU. In her thesis she is investigating the influence of hypoxia in delayed wound healing of the skin and specifically on primary keratinocytes and fibroblasts after exposure to sulfur mustard. This project is supported by the German Federal Ministry of Defense.

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