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Effects of sulfur mustard on mesenchymal stem cells

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ABSTRACT

Chronic wound healing disorders that occur as a result of a sulfur mustard (SM) exposure present a particular challenge. These chronic wounds are similar to other chronic wounds. In the past, it has been shown that mesenchymal stem cells (MSC) play an important role in the healing of chronic wounds. An important property to support wound healing is their ability to migrate. However, we were able to show that SM leads to a reduction in MSC migration even at low concentrations. Currently, exposed MSCs are still able to differentiate. Further alterations are not known. The current investigation therefore focused onto the question how SM affects MSC.
Material & methods: The effect of SM on MSC was investigated. Here, the alkylation of DNA was considered, and DNA adducts were quantified over a period of 48 h. The modification of the nuclei under the influence of SM was analyzed as well as proliferation of the cells by immunohistochemical staining with Ki-67 and quantification. For the quantification of the apoptosis rate, antibodies against cleaved Caspase-3, 8, and apoptosis inducing factor (AIF) were used. The senescence analysis was performed after histological staining against β-galactosidase. Quantifications were carried out by using the TissueQuest System and the software TissueFAX.
Results: SM exposure of MSC results in a dose dependent formation of nuclear DNA adducts. 4 h after exposure the cells display a decreasing concentration of DNA adducts. This process is accompanied by a change of nuclei shape but without an increase of apoptosis induction. In parallel the number of cells undergoing senescence increases as a function of the SM concentration.
Discussion: SM exposure of MSC leads to adduct formation on chromosomal DNA. These DNA adducts can be reduced without MSC are undergoing apoptosis. This indicates an active DNA damage response (DDR) pathway in combination with the formation of persistent nuclear DNA damage foci. This process is accompanied by a reduced capability of proliferation and a transition into the senescent state.

1. Introduction

Mesenchymal stem cells seem to be a useful tool for the regeneration and healing of wounded tissue. Various approaches in the field of tissue engineering have been tested in recent years using mesenchymal stem cells. MSCs have the ability to differentiate into different cell types (Pittenger et al., 1999; Muraglia et al., 2000) and rebuild, for example, bone tissue (Meinel et al., 2004), cartilage tissue (Li et al., 2005), or vessels (Koike et al., 2004). MSC are not only able to reconstruct a tissue, they also have the ability to control or cure other tissues by coordinating the reconstruction process (Schinkothe et al., 2008). It is known that normal skin is a target organ for bone marrow–derived cells from both the hematopoietic and the mesenchymal stem cell pool. Bone marrow derived cells demonstrate a strong contribution to normal skin and the healing of cutaneous wounds (Fathke et al., 2004).

As a result of tissue injury, hematopoietic and mesenchymal stem cells are mobilized in the bone marrow and enter the circulation. The cells migrate from there into the injured tissue, where they regulate the proliferation and migration of epithelial cells and dermal stroma cells during the early inflammatory phase (Singer and Clark, 1999). Moreover it is known that bone marrow derived MSC of patients with chronic wounds demonstrate a significant reduced migratory attraction effect to skin fibroblasts compared with MSC derived from healthy donors (Rodriguez-Menocal et al., 2012).

Sulfur mustard (SM) has now a history of hundred years since its first use as a chemical warfare agent in World War I 1917 in Ypres (Pechura and Rall, 1993). After contact with SM, injuries to the skin are likely to occur, but also the eyes and respiratory tract can be affected.

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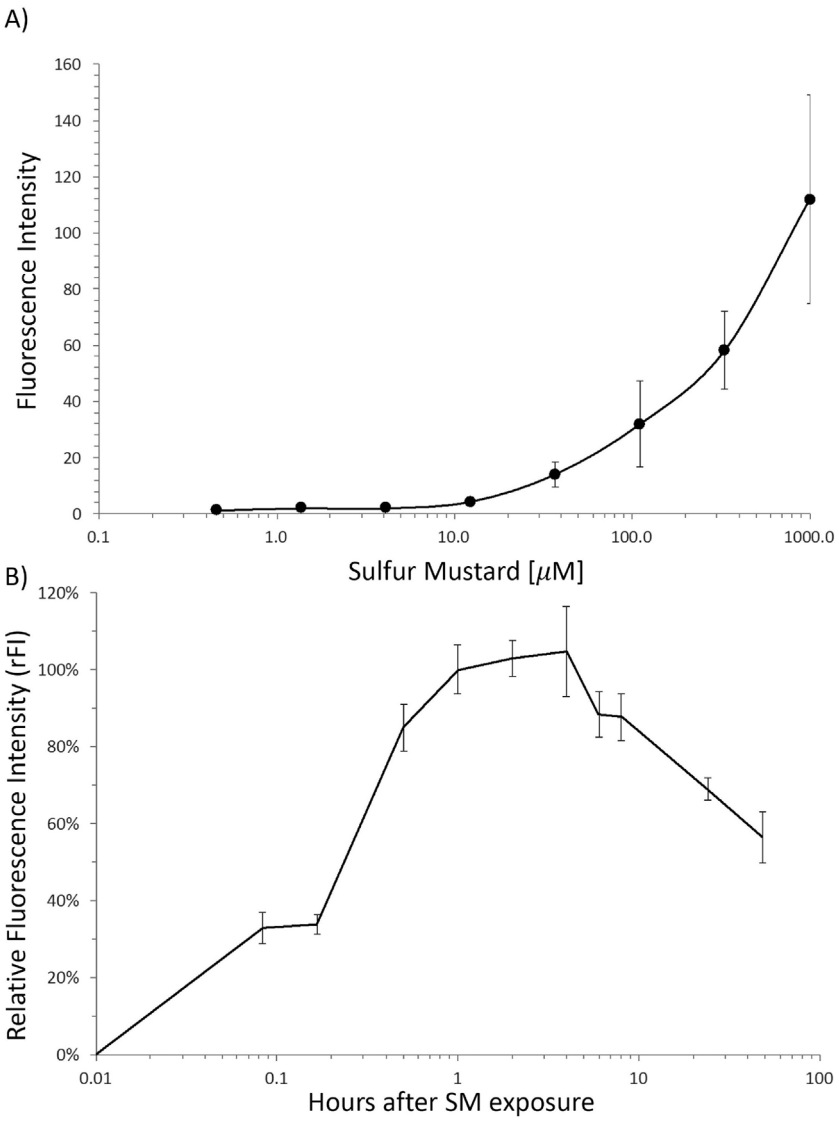

Fig. 1. Detection of SM-induced DNA adducts. SM induced DNA adducts can be detected with the antibody 2F8. A) An increase in fluorescence intensity reflects the increase in the amount of DNA adducts. A continuous increase of the DNA adducts under increasing SM concentration could be observed. B) An investigation into the time dependence of the DNA adducts under the influence of 100 µM SM showed a continuous increase of the adducts up to an hour after exposure. Between one and four hours a plateau phase took place. After four hours, the adducts decrease to the end of observation after 48 h.

Skin lesions are seen in more than 90% of patients exposed to SM (Emadi et al., 2012, 2009, 2008).

After exposure to the skin, clinical symptoms occur with a delay of 2–14 h depending on dose, temperature, moisture, and the anatomical site of exposure.

The most devastating aspect of SM exposure is that wound healing occurs over a prolonged time period (chronic wounds) as compared to other blister-forming injuries resulting from, for example ultraviolet (UV) light exposure (Graham et al., 2005).

MSC: are known to be highly resistant against SM. Already very low concentrations of SM demonstrate a strong effect on the migratory activity, whereas the differentiation capacity is not affected (Schmidt et al., 2013).

It is therefore very likely that a link between MSC and the impaired wound healing after SM exposure exists. However, the underlying mechanisms of SM resulting in MSC dysfunction are unknown and are addressed in the present study.

2. Material and methods

2.1. Cell culture

MSC: of human origin were obtained from bone marrow aspirates, as well as from bone marrow from femoral head. Material from 10 patients was used. At the time of sampling, patients were between 49 and 84 years, with a mean age of 66.2 years. Only patients in good health conditions (except femoral head fracture) were chosen as donors. Especially patients with severe diseases like cancer and after chemotherapy were excluded. Before preparing Ficoll-Paque PLUS density gradient centrifugation (Amersham Biosciences, Uppsala, Sweden), the bone marrow was diluted with PBS and filtered (70 µm mesh). The concentrated cells were washed several times before plating them in a plastic petri dish. First change of medium (α-minimal essential medium, 20% [v/v] fetal calf serum [FCS], 200 µM l-glutamine, 100 U/ml penicillin, 100 U/ml streptomycin) was performed 2 days after culturing (95% humidity, 5% CO_2). Cells were used until passage 3. For every passage or experiment, the cells were plated at 2000 cells per cm^2. The medium was changed twice a week. The study was approved by the ethics committee of the Ludwig-Maximilians-Universität Munich, Germany and conforms to the Declaration of Helsinki.

2.2. MSC: culture quality control

The quality of cultured MSC was controlled by microscopic assessment of the morphology, flow cytometry, CFU-F assay, and differentiation assays as described previously (Schmidt et al., 2006).