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# Impact of topical application of sulfur mustard on mice skin and distant organs DNA repair enzyme signature

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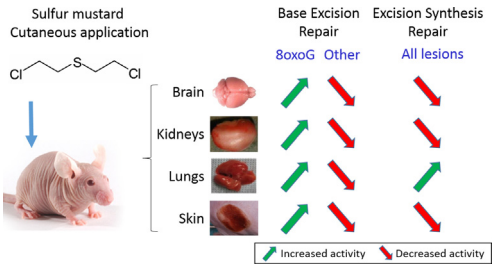
## HIGHLIGHTS

- Topical application of sulfur mustard affects DNA repair in skin and internal organs.
- Each organ is differently affected as soon as 4 h after application of SM.
- Most glycosylase/AP endonuclease activities decrease but repair of 8oxoG increases.
- DNA excision/synthesis activities are inhibited in skin, kidneys and brain.
- Most DNA excision/synthesis activities are enhanced in lungs after SM exposure.

## GRAPHICAL ABSTRACT

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## ABSTRACT

Sulfur mustard (SM) is a chemical warfare agent that, upon topical application, damages skin and reaches internal organs through diffusion in blood. Two major toxic consequences of SM exposure are inflammation, associated with oxidative stress, and the formation of alkylated DNA bases. In the present study, we investigated the impact of exposure to SM on DNA repair, using two different functional DNA repair assays which provide information on several Base Excision Repair (BER) and Excision/Synthesis Repair (ESR) activities. BER activities were reduced in all organs as early as 4 h after exposure, with the exception of the defense systems against 8-oxo-guanine and hypoxanthine which were stimulated. Interestingly, the resulting BER intermediates could activate inflammation signals, aggravating the inflammation triggered by SM exposure and leading to increased oxidative stress. ESR activities were found to be mostly inhibited in skin, brain and kidneys. In contrast, in the lung there was a general increase in ESR activities. In summary, exposure to SM leads to a significant decrease in DNA repair in most organs, concomitant with the formation of DNA damage. These synergistic genotoxic effects are likely to participate in the high toxicity of this alkylating agent. Lungs, possibly better equipped with repair enzymes to handle exogenous exposure, are the exception.

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## 1. Introduction

Sulfur mustard (SM) is a chemical warfare agent which has been used for almost a century, first in World War I and more recently in Iraq (Balali-Mood and Hefazi, 2006) and Syria. SM is also considered as a potential chemical weapon for terrorist actions (Smith et al., 1995; Wattana and Bey, 2009). Consequently, SM remains a threat for both militaries and civilians. SM has three main targets in humans, namely eyes, lungs, and skin (Balali-Mood and Hefazi, 2005). In the latter organ, exposure to SM leads to the formation of large blisters which poorly heal (Momeni et al., 1992; Ghanei et al., 2010). SM was consequently classified as a vesicating agent. Damage to organs and in particular in skin results from a massive cell death by necrosis and apoptosis. In addition an acute inflammatory response takes place. At the molecular level, these deleterious properties are explained by the alkylating properties of SM which efficiently reacts with biomolecules.

Induction of DNA damage is proposed to be strongly involved in the lethal process (Papirmeister et al., 1985; Debiak et al., 2009; Kehe et al., 2009). Indeed, reaction of SM with DNA leads to the formation of a wide array of damage including monoadducts to guanine and adenine, inter- and intra-strand guanine biadducts (Brookes and Lawley, 1960, 1961, 1963; Fidder et al., 1994; Ludlum et al., 1994) and a recently characterized ternary glutathione-SM-guanine adduct (Batal et al., 2015). In addition exposure to SM results in GSH depletion and oxidative stress which in turn induces oxidative DNA lesions (Pal et al., 2009; Tewari-Singh et al., 2012).

Information on the *in vivo* formation of SM-DNA adducts have mostly been obtained following cutaneous exposure using either immunological detection (van der Schans et al., 2004) or HPLC-mass spectrometry quantification (Batal et al., 2013; Yue et al., 2014). Adducts were found to be readily produced, even at low doses. In addition, they are quite persistent and could be detected in skin as long as three weeks after exposure. Interestingly, even after cutaneous exposure, SM diffuses through the skin, reaches the blood and then internal organs (Cullumbine, 1946; Chilcott et al., 2000; Goswami et al., 2015; Yue et al., 2015). Among other consequences, DNA adducts are produced (Batal et al., 2014; Yue et al., 2015) with brain and lungs being the most sensitive targets.

In contrast to the formation of SM adducts, little is known about their repair. Experiments in a series of genetically deficient cells showed that nucleotide excision repair (NER), a repair mechanism handling both bulky lesions and interstrand crosslinks, was important for the repair of SM-induced DNA damage and cell survival in eukaryotes (Kircher et al., 1979; Matijasevic et al., 2001; Matijasevic and Volkert, 2007; Jowsey et al., 2012). In bacteria, evidence were also obtained for a role of both repair of interstrand crosslinks (Lawley and Brookes, 1965) and of 3-methyladenine DNA glycosylase II (Matijasevic et al., 1996). Moreover, SM-DNA adducts are unstable and can depurinate, leading to the formation of abasic sites. This class of DNA lesions is efficiently repaired in cells by AP endonucleases such APE1 (Hoeijmakers, 2001; Robertson et al., 2009). In spite of these biochemical data, no information is available on the impact of exposure to SM on the overall DNA repair efficacy and on the modulation of the different repair pathways in cells.

We designed the present study to address this latter point in order to obtain mechanistic information and propose a possible biomarker of exposure. SKH-1 hairless mice were exposed to SM on the back. DNA repair was studied in skin, brain, lungs and kidneys, the organs where the level of DNA adducts was found to be the highest in a previous study (Batal et al., 2013, 2014). DNA repair activities were quantified using two different functional DNA repair assays developed on biochips (Millau et al., 2008; Pons et al., 2010). The ODN biochip, a multiplexed version of the Oligonucleotide (ODN) Cleavage Assay, provided information on the glycosylase activities associated with the bases excision repair pathway (BER), against a series of modified bases incorporated into different ODNs. The plasmid microarray, functionalized with series of plasmids containing different small or bulky lesions, allowed us to quantify excision/synthesis repair (ESR) activities and thus was relevant to both NER and BER. These two techniques were applied to whole protein extracts from the four investigated organs and showed that SM actually modulates DNA repair activities whatever the organ investigated, through a direct impact and possibly in relation to inflammation.

## 2. Materials and methods

### 2.1. Mice exposure to SM

Male, euthymic and hairless SKH-1 mice (Crl: SKH1-hr, 4–6 weeks of age) were purchased from Charles River Laboratories (L'Arbresle, France). They were housed and acclimatized for one week before experiment with food and water *ad libitum*. Treatment was made as described elsewhere (Batal et al., 2013). All procedures were in accordance with the regulations regarding the "protection of animals use for experimental and other scientific purposes" from the relevant Directives of the European Community (86/606/CE). Study protocols were approved by the Ethical Committee of the French Armed Forces Biomedical Research Institute. Briefly, on the day of exposure, animals were anesthetized (ketamine hydrochloride and diazepam) and randomly assigned to treatment groups ($n = 3$). For pain relief, buprenorphin (0.05 mg/kg) was delivered by a subcutaneous injection. SM, diluted in 2 μL of dichloromethane, was topically applied on an ink-marked circular zone of 0.28 $cm^2$ on the dorsal–lumbar region of the animal (Dorandeu et al., 2011). Animals were exposed to SM dose of either 0.6 or 6 mg/kg. SM was removed from the skin after 4 h using 0.8% sodium hypochlorite and natural sponges. Four hours or 24 h after the end of the exposure, mice were euthanized by intraperitoneal injection of a lethal dose of pentobarbital (100 mg/kg). Brain, lungs, kidneys and skin punches (8 mm diameter at the treated site) were rapidly collected and processed immediately for extract preparation. The mice identification numbers and the corresponding treatment conditions are listed in Table S1.

### 2.2. Preparation of whole cell extracts from mice organs

The fresh organs were cut into small slices, and placed in 2 mL of ice-cold HEPES/KOH 90 mM pH 7.8, KCl 0.8 M, EDTA 2 mM, glycerol 20%, DTT 1 mM. They were then disrupted and homogenized for 30 s at medium speed using the Qiagen TissueRuptor®. The lysis was completed by one cycle of freezing/thawing in liquid nitrogen and 4 °C, respectively. A second ice-cold buffer (150 μL) was subsequently added (HEPES/KOH 45 mM pH 7.8, EDTA 0.25 mM, glycerol 2%, 5 mM phenylmethane sulfonyl fluoride) before a second round of freezing/thawing. Lastly the lysates were cleared by 5 min centrifugation at 16 000 g at 4 °C and stored frozen in 100 μL aliquots at −80 °C. The protein concentration was determined in each sample using the BCA kit (Interchim, Montluçon, France).

### 2.3. DNA repair assays

#### 2.3.1. Multiplexed ODN cleavage assay

A panel of DNA duplexes, each containing a different lesion repaired by BER, were immobilized at specific sites on glass slides, forming 24 identical pads. On each pad, a control ODN (Lesion_Free ODN) and eight lesion-containing ODNs were available in

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