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Medical documentation, bioanalytical evidence of an accidental human exposure to sulfur mustard and general therapy recommendations

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HIGHLIGHTS

- The vesicant sulfur mustard (SM) is a banned chemical warfare agent.
- 3 patients were accidentally exposed to SM vapor developing erythema and blisters.
- Bioanalytical mass spectrometry-based methods allowed verification of exposure.
- Full recovery was observed under symptomatic therapy on day 56 after exposure.
- General recommendations for therapy and management of poisoning are given.

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ABSTRACT

Sulfur mustard (SM) is a chemical warfare agent (CWA) that was first used in World War I and in several military conflicts afterwards. The threat by SM is still present even today due to remaining stockpiles, old and abandoned remainders all over the world as well as to its ease of synthesis. CWA are banned by the Chemical Weapons Convention (CWC) interdicting their development, production, transport, stockpiling and use and are subjected to controlled destruction. The present case report describes an accidental exposure of three workers that occurred during the destruction of SM. All exposed workers presented a characteristic SM-related clinical picture that started about 4 h after exposure with erythema and feeling of tension of the skin at the upper part of the body. Later on, superficial blister and a burning phenomenon of the affected skin areas developed. Similar symptoms occurred in all three patients differing severity. One patient presented sustained skin affections at the gluteal region while another patient came up with affections of the axilla and genital region. Fortunately, full recovery was observed on day 56 after exposure except some little pigmentation changes that were evident even on day 154 in two of the patients.

SM-exposure was verified for all three patients using bioanalytical GC MS and LC MS/MS based methods applied to urine and plasma. Urinary biotransformation products of the β-lyase pathway were detected until 5 days after poisoning whereas albumin-SM adducts could be found until day 29 underlining the beneficial role of adduct detection for post-exposure verification. In addition, we provide general recommendations for management and therapy in case of SM poisoning.

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1. Introduction

Sulfur mustard (SM, bis(2-chloro-ethyl) sulfide, CAS No. 505-60-2) is a chemical warfare agent (CWA) first used during World War I (WWI) causing severe and painful lesions. The clinical picture of sulfur mustard poisoning is well known especially from the thousands of victims from WWI and the Iran–Iraq war of the 1980 years (Haines and Fox, 2014). Ocular, dermal and pulmonary symptoms occur frequently after contact to the agent while affection of the hemopoetic system is associated with a high dose exposure (Kehe and Szinicz, 2005). Symptoms develop with a characteristic time delay in which the onset of symptoms is depending on the SM concentration and exposure time. Higher doses will result in a shorter delay until symptoms occur. SM can affect skin, eyes, lungs and can cause systemic effects. Dermal symptoms are most common and are characterized by erythema, burning sensations, itching, vesication, ulceration, wound healing disorder and pigmentation disorder. Skin areas with increased moisture (e.g., axillae, elbow, scrotum, anal region) are more sensitive to SM (Kehe and Szinicz, 2005). Vapor exposure usually results in extensive skin damage whereas contact to liquid SM induces local dermal affections. For vapor exposure a threshold dose of 100–300 mg * min/m^3 has been described (Kehe and Szinicz, 2005). Dermal symptoms will occur 4–8 h after exposure and start with itching and erythema. Blister formation is likely to occur at vapor doses of 1000–2000 mg * min/m^3 (Kehe and Szinicz, 2005). A positive Nikolsky phenomenon (dislodgement of intact superficial epidermis by a shearing force, indicating a plane of cleavage in the skin) is common (Braue et al., 1997). Affected skin areas often present a distinct tanning that can persist for decades or can change into hypopigmentation (Kehe and Szinicz, 2005). Healing of affected skin areas is slow and requires several weeks to months.

SM became a widely used chemical even in the post-WWI era. The threat by this CWA is still present due to its ease of synthesis as well as to remaining stockpiles and abandoned ammunition all over the world. CWA are banned by the Chemical Weapons Convention (CWC) interdicting their development, production, transport as well as stockpiling and use. In addition, a main objective of the CWC is the destruction of all chemical weapons, a process that is controlled by the Organisation for the Prohibition of Chemical Weapons (OPCW, 2015 www.opcw.org). Accordingly, the investigation of an alleged use of CWA potentially leading to poisoned victims requires bioanalytical methods for forensic analysis proving any violations of law. Moreover, in case of an accidental exposure during destruction of such compounds reliable methods for the post-exposure *in vivo* analysis are also relevant to document incorporation of the poison. Bioanalytical methods -typically based on gas (GC) and liquid chromatography (LC) in combination with selective mass spectrometry—are required to prove SM exposure targeting diverse SM-derived analytes. *In vivo* SM undergoes (i) rapid non-enzymatic hydrolysis to thiodiglycol (TDG), (ii) ß-lyase catalyzed transformation to 1-methylsulfinyl-2-[2-(methylthio) ethylsulfonyl]ethane (MSMTESE) and 1,1′-sulfonylbis-[2-(methylsulfinyl) ethane] (SBMSE) after activation by glutathione, and (iii) reaction with DNA and endogenous proteins forming alkylated adducts (John et al., 2015a). SM itself as well as its hydrolyzed and enzymatically converted products are rapidly degraded and excreted from the body within hours to days. The resulting short *in vivo* half-life thus represents a major challenge for instrumental bioanalytical verification especially when sample drawing is done several days after exposure. In contrast, protein adducts exhibit much longer half-lives of some weeks to months thus being beneficial for successful post-exposure analysis (John et al., 2015a). The adduct of human albumin (HSA) produced by reaction of SM with its only free cysteine residue (Cys^{34}) represents a well-known marker for verification analysis allowing adduct detection up to 4 weeks after exposure (Noort et al., 2008). Very recently, a novel method was developed and validated at the Bundeswehr Institute of Pharmacology and Toxicology (InstPharmToxBw) to detect this albumin adduct in human plasma (Gandor et al., 2015; John et al., 2015b). InstPharmToxBw is the only German national center of competence concerned with all aspects of medical chemical defense (Med C-defense). Scientific research is focused on e.g., discovery of novel drug lead structures, therapy optimization and bioanalytical verification of poisoning with chemical warfare agents and related toxicants (John and Thiermann, 2012).

Here we describe an accidental exposure of three workers that occurred during the destruction of SM hydrolysate mixtures. All patients gave consent to publish their photos and data.

2. Case presentation

SM hydrolysates stored in barrels were to be burned in a special combustion furnace following an established routine procedure. Barrels were disposed on a lorry and were brought into the furnace. A mechanical malfunction let a team of three workers decide to enter the vestibule of the combustion furnace to restore the proper progress of the combustion process. All workers were wearing a protective mask, gloves, boots and a dust protection dress and left the vestibule after some 15–20 min.

About 4 h after that event all workers recognized a tension phenomenon of the skin at the upper part of the body. Later on, erythema and skin affections developed. All three patients presented similar symptoms that differed in severity.

Patient 1 (male, 32 years, good health conditions) recognized a burning sensation and erythema 4 h after the incident and visited a local hospital on the evening of the incident. A treatment with cortisone containing ointment was initiated. The day after the patient turned to the occupational medicine department and was treated locally with silver sulfadiazine (Flammazine® lotion) and diclofenac systemically. On day 15 after exposure an out-patient examination at a specialist hospital revealed first-degree affections (erythema) of the entire ventral and dorsal upper part of the body, the neck, forehead, arms including elbows and gluteal region (Fig. 1A). At the gluteal region (Fig. 1B), axillae and partially at the upper part of the body second-degree affections (skin ablation) were observed. In total 39% of the body surface was affected. The patient reported at an out-patient examination on day 20 that pruritus (itchiness) was still existing, but less intense. Treatment with dexpanthenol (panthenol) decreased the tension sensations. The erythema and overall conditions improved distinctively. At the following out-patient examination on day 29 the patient reported temporarily pruritus, hypersensitivity and burning sensation in the formerly affected areas. In addition, increased sweating especially at the elbow regions was noticed by the patient. A distinct hypopigmentation of the affected areas was observed. At this time, no open wounds were obvious. At the out-patient examination on day 56 after exposure almost full recovery (restitutio ad integrum) was achieved (Fig. 1A,B). The patient only reported occasional pruritus but which did not restrict the patient. Ocular and pulmonary symptoms were not evident at any point in time. At the final out-patient examination on day 154 after exposure only some little remaining hyperpigmentation of the elbow regions were observed.

Blood samples were taken on days 3, 5, 13, 15, 20, 29, and 103 and urine samples were collected on days 3, 5, 13, and 15 after exposure and were sent for forensic analysis to the InstPharmToxBw.

Patient 2 (male, 48 years, good health conditions) recognized a sunburn-like sensation 2.5 h after the incident. The day after the patient turned to the occupational medicine department and was