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Short Communication

EFFICACY OF MINERAL CATIONIC CARRIER AGAINST SULPHUR MUSTARD IN SKIN DECONTAMINATION

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The aim of this study was to evaluate decontamination (absorption) efficacy of a preparation called Mineral Cationic Carrier (MCC®) against skin contamination with sulphur mustard *in vivo*. MCC® is a synthetic preparation with known ion exchange, absorption efficiency, and bioactive potential. CBA mice were applied increasing doses of sulphur mustard on their skin and MCC® was administered immediately after skin contamination. The results have confirmed the decontamination efficacy of MCC® preparation, corresponding to 8.4 times the LD_{50} of percutaneous sulphur mustard, and call for further investigation.

KEY WORDS: *CBA mice, MCC®, decontamination efficacy, yperite, warfare agent*

Sulphur mustard (yperite) is a liquid blister agent, which is colourless when pure. It is insoluble in water and very difficult to decontaminate. Surface decontamination may be accomplished using hypochlorite bleach slurries, STB, or DS2 decontaminating solution (1-3). The effects of sulphur mustard on the skin should be considered in terms of exposure to the agent in both liquid and vapour from. Liquid exposure to 50 μg cm^{-2} for 5 min causes a slight erythema, and exposure to (250 to 500) μg cm^{-2} for 5 min leads to blistering (4). The precise percutaneous LD_{50} of sulphur mustard is not known in humans, but death is said to have occurred on exposure to 64 mg kg^{-1}. In rats, percutaneous LD_{50} is 9 mg kg^{-1}, and in dogs and rabbits 20 mg kg^{-1} and 100 mg kg^{-1}, respectively (5).

Animal studies have shown that mustard is mutagenic and carcinogenic. Sulphur mustard has a direct effect on DNA and is occasionally effective after a single exposure (5, 6).

Possible contamination of skin by mustard agents stresses the urgency of effective primary decontamination of naked skin (7).

In the search for a new decontaminant, three main directions were followed: a) decontamination solutions and emulsions; b) decontamination ointments, gels, and pastes, and c) decontamination adsorption and chemisorption powders (8).

Many of the preparations for decontamination against sulphur mustard are toxic. However, military personal decontamination kits include substances and preparations which are non-toxic, ecologically acceptable, and safe (9-11).

The aim of this *in vivo* study was to evaluate decontamination efficacy of a preparation called Mineral Cationic Carrier (MCC®) in mice treated with increasing doses of sulphur mustard on skin (1, 2).

MCC® is a synthetic powder preparation with known ion exchange, absorption efficiency, and bioactive potential (12, 13). Preclinical toxicology of the preparation was done according to the standards and regulations of the Organization for Economic Cooperation and Development (OECD) and showed no acute, sub-chronic, or chronic toxicity (12).

Due to these biochemical properties, present study was the first to use MCC® as a skin decontaminant against a blister warfare agent.

MATERIAL AND METHODS

Chemicals

Sulphur mustard [HD, bis(2-chloroethyl) sulfide, $C_4H_8Cl_2S$] was used in compliance with the Chemical Weapons Convention (14). It was obtained from the Laboratory for NBC Protection and Biomonitoring (Zagreb, Croatia).

MCC® (patent HR2002/000034) is a calcium/sodium (30:70) powder synthesised at the Ruđer Bošković Institute (Zagreb, Croatia), and manufactured by KODONA (Zagreb, Croatia). The synthesis of MCC from aluminosilicate hydrogels involved a chain of procedures. The synthetic zeolite consists of a sodium form $Na_2O{\bullet}Al_2O_3{\bullet}ySiO_2{\bullet}z\,H_2O$ (y=2-50 and z=1.5-6) as a fine white powder. A calcium form was obtained by suspension in a solution of calcium ions using the standard procedure of ion exchange (12).

In vivo *experiments*

Mice (CBA strain) were purchased from the Ruđer Bošković Institute. The experiment was done on adult, 12 to 30-week-old animals of both sexes, weighing 28 g to 32 g. Before the experiments started, the animals had free access to food and water and were kept under conventional conditions.

The study was carried out according to the NIH Guide for the Care and Use of Laboratory Animals. Experiments received necessary approvals from the Local Ethical Committee. Immediately before receiving sulphur mustard, the animals were sedated with 35 mg kg^{-1} b. w. pentobarbital sodium (Nembutal®, Abbot Lab., Chicago, USA).

Sulphur mustard was used for contamination as a chemical warfare agent, and was administered on the shaved back skin of the animals using a pipetman by Eppendorf.

About 2 g of MCC® powder per animal was scrubbed on the same site one minute after contamination (intoxication) with sulphur mustard. Four mice received a supralethal dose and six a sub-lethal dose. Experiments were repeated twice.

To determine mortality rate in following 24 hours, animals that survived the experiment were returned to cages and kept in conventional conditions. After 24 hours the surviving animals were sedated and sacrificed.

Statistical evaluation of the results in vivo

The lethal doses (LD_{50}) and 95 % limit of reliability (L_R) were calculated from Weil's tables (15) using the following equations:

$$\log LD_{50} = \log D_A + \log G_f\,(1+f)$$
$$\log 95\,\%\ L_R = \log LD_{50} \pm 2d \times \sigma$$

The therapeutic decontamination effect (TDE) of MCC® was calculated using the following equation:

TDE = LD_{50} (with decontamination) / LD_{50} (without decontamination)

RESULTS AND DISCUSSION

Decontamination against a chemical warfare agent mustard should be efficient, toxicologically safe, and environment friendly (11, 16, 17). Sorbent decontaminants are nontoxic, free-flowing, solid materials which absorb a liquid agent. They are used by a soldier to wipe bulk liquid agent from the skin, clothing, and personal equipment (1, 2, 13, 18). This study investigated for the first time the efficacy of MCC® as skin decontaminant against a sulphur mustard using methods similar to earlier research (19, 20). MCC® is like a solid sorbent system. It is a synthetic substance, with known ion exchange, absorption efficiency, and bioactive potential (12, 13, 21). Three-dimensional net structure and micro-size inorganic particles largely contributed to its absorption properties.

Table 1 shows the survival of mice receiving sulphur mustard, but not the MCC® antidote while Table 2 shows the survival of mice receiving supra-lethal doses of sulphur mustard and immediately treated with MCC®.

The lethal dose (LD_{50}) of percutaneous sulphur mustard in CBA mice (Table 1) was 497.90 mg kg^{-1} at 95 % limit of reliability (95 % L_R) (5) (Table 3). The survival of mice given eight times the LD_{50} of percutaneous sulphur mustard and decontaminated with MCC® preparation suggests that MCC® is very efficient in decontamination (Table 2 and 3) and absorption (2, 3, 18).

Sidell and Hurst (9) have described the long-term clinical effects of acute symptomatic exposure to sulphur mustard, but less is known about the clinical effects of chronic, sometimes symptomatic, low-dose exposure. Sulphur mustard in doses of 537.8 mg kg^{-1} and 677.7 mg kg^{-1} caused mortality in 66 % (4/6) and 100 % (6/6) of exposed, but untreated

Table 1 *The survival of mice (CBA strain) receiving LD_{50} sub- and supra-lethal doses of percutaneous sulphur mustard which were not treated with MCC®*

Sulphur mustard doses / mg kg^{-1}	Volume per mouse / mL	D/C
338.7	0.80	0/6
426.8	1.01	1/6
537.8	1.27	4/6
677.7	1.60	6/6

D - number of dead mice, C - number of contaminated mice

Table 2 *The survival of mice (CBA strain) receiving supra-lethal doses of percutaneous sulphur mustard which received skin treatment with the MCC® preparation*

Sulphur mustard doses / mg kg^{-1}	Volume per mouse / mL	D/C
2963.3	7.00	0/4
3733.7	8.82	1/4
4704.5	11.11	3/4
5927.7	14.00	4/4

D - number of dead mice, C - number of contaminated mice

Table 3 *Skin decontamination against sulphur mustard determined in vivo*

	Sulphur mustard skin poisoning		
	LD_{50} / mg kg^{-1}	95 % L_R / mg kg^{-1}	TDE
Without decontamination	497.90	(433.98-571.12)	/
With decontamination	4191.79	(3540.05-4908.97)	8.4

TDE - *Therapeutic decontamination effect*
L_R-*95 % Limit of reliability*

animals, respectively (Table 1). It is important to note that the MCC prevented death in mice exposed to concentrations as high as 2963.3 mg kg^{-1} (Table 2) and to significantly reduce their death rate at higher mustard doses.

In this study the sub-lethal and supra-lethal percutaneous doses of sulphur mustard were applied to large number of mice, both male and female. Preliminary results showed similar results for both sexes.

CONCLUSIONS

Animals which were decontaminated by the MCC® preparation survived and recovered from as high as 8.4 times the lethal dose of percutaneous sulphur mustard. The recovered animals had no changes on their skin, which was confirmed 24 hours later. These results suggest that MCC® is a very efficient decontaminant against sulphur mustard.

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Sažetak

DJELOTVORNOST PRIPRAVKA MCC® KAO KOŽNOG DEKONTAMINANTA SUMPOROVA IPERITA

Cilj ovog istraživanja bio je ispitati dekontaminacijska (adsorpcijska) svojstva pripravka MCC® (*Mineral Cationic Carrier*) rabeći kožni bojni otrov iperit kao kontaminant u uvjetima *in vivo*.
MCC® je sintetski pripravak koji je biokemijski aktivan i ima ionskoizmjenjivačka i adsorpcijska svojstva. Istraživanje u uvjetima *in vivo* provedeno je na miševima (soj CBA), aplikacijom rastućih doza iperita na kožu životinje. Pripravak MCC® uporabljen je kao kožni dekontaminant neposredno nakon perkutane kontaminacije iperitom.
Rezultati istraživanja pokazuju da se dekontaminacijom pripravkom MCC® može postići terapijski učinak od 8,4 LD_{50} (perkutano, iperit).
Dobiveni rezultati potvrđuju vrlo dobru dekontaminacijsku učinkovitost pripravka MCC® i govore u prilog daljnjim istraživanjima s ciljem njegove moguće šire primjene.

KLJUČNE RIJEČI: *bojni otrov, dekontaminacija kože, miševi CBA, terapijski učinak*

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