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Short Communication

SKIN DECONTAMINATION WITH MINERAL CATIONIC CARRIER AGAINST SARIN DETERMINED *IN VIVO*

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Our Institute's nuclear, biological, and chemical defense research team continuously investigates and develops preparations for skin decontamination against nerve agents. In this *in vivo* study, we evaluated skin decontamination efficacy against sarin by a synthetic preparation called Mineral Cationic Carrier (MCC®) with known ion exchange, absorption efficacy and bioactive potential. Mice were treated with increasing doses of sarin applied on their skin, and MCC® was administered immediately after contamination. The results showed that decontamination with MCC® could achieve therapeutic efficacy corresponding to 3 x LD_{50} of percutaneous sarin and call for further research.

KEY WORDS: *bioactive potential, MCC®, nerve agent, NOD mice, sorption powder, toxicity*

Organophosphate (OP) nerve agents (G-agents and VX) are related to the OP pesticides, but have a much higher mammalian acute toxicity, particularly through skin and inhalation route (1-4).

Warfare nerve agent sarin is a codename suggested by Schrader as a derivation of Schrader, Ambros, Rüdriger and Van der Linde, who synthesised the compound. Its acute effects are primarily due to unrestricted cholinergic activity at both muscarinic and nicotinic receptors (1, 3-5). The threat of skin contamination by nerve agents calls for effective primary decontamination preparation for naked skin (6). Developing effective treatment and decontamination systems has proven to be a demanding task. Not only do the varying chemical properties of nerve agents impede the development of a single, "universal" decontaminant, but the extreme toxicities of these agents necessitate a matching counter-efficiency. Searching for a new decontaminant, we focused on the three main forms: 1. decontamination solutions and emulsions, 2. decontamination ointments, gels and pastes; and 3. decontamination adsorption and chemisorption powders (7).

However, many of the preparations for skin decontamination against nerve agents are toxic. Accordingly, army-issue personal decontamination kits include substances and preparations which are non-toxic, ecologically acceptable and safe (2, 8, 9). Previous research indicated that a synthetic powder Mineral Cationic Carrier (MCC®), otherwise used as a dietary supplement, possesses ion exchange, absorption, and bioactive potential to counteract skin contamination by nerve agents (10-12).

Preclinical toxicology testing performed according to the standards and regulations of the Organization for Economic Cooperation and Development (OECD) did not indicate acute, sub-chronic, or chronic toxicity of the preparation (11), and it proved efficacious against skin contamination with warfare nerve agent sulphur mustard (13). In this study, we investigated the efficacy of MCC® against skin contamination with sarin.

MATERIAL AND METHODS

Chemicals

Sarin was used as a contaminant in accordance with the Chemical Weapons Convention (14). The compound (isopropyl methyl phosphonofluoridate, $C_4H_{10}PO_2F$) was obtained from the Laboratory for NBC Protection and Biomonitoring (Zagreb, Croatia). Solution of sarin (c=100 mg L^{-1}) was prepared in isopropanole (Sigma, USA).

MCC® (patent HR2002/000034) is a calcium/sodium (30:70) powder preparation synthesised at Ruđer Bošković Institute (Zagreb, Croatia) and manufactured by KODONA (Zagreb, Croatia). Details about its form and synthesis are given elsewhere (11, 13).

In vivo *experiments*

Mice (NOD strain) were purchased from Ruđer Bošković Institute. The experiment included adult animals of both sexes at approximately 12 to 30 weeks of age and 28 to 32 g of weight. Before the experiment, the animals were kept under conventional conditions and were receiving food and water *ad libitum*.

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 Ethics Committee. The day before the experiment, the mice were marked, weighed, shaved using electric shaver, and divided in groups of four. Immediately before receiving sarin, they were sedated with 35 mg kg^{-1} b. w. pentobarbital sodium solution (Nembutal®, Abbot Lab., Chicago, USA). Sarin was administered on the shaved back skin of the animals in increasing volumes of (0 to 1.4) mL using a pipetman by Eppendorf. About 2 g of MCC® powder was sprinkled over the same site and rubbed in with a spoon one minute after contamination with sarin. The efficacy of the preparation was tested on four mice per dose and 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.

Median lethal sarin dose (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

MCC® is a synthetic zeolite with a three-dimensional net structure and known ion exchange, absorption efficiency, and bioactive potential (9-12, 19-22). Our earlier study on CBA has shown that it is also an efficacious skin decontaminant against high doses of sulphur mustard (13). This study was performed on a similar *in vivo* model (NOD mice) to evaluate the efficacy of *MCC®* against chemical warfare agent sarin. We applied the same array of methods as proposed in earlier research (16-19).

The median lethal dose of percutaneous sarin for NOD mice is 1221.80 μg kg^{-1}. According to literature data (17) it is within the 95 % limit of reliability (95 % L_R) (Table 1). Animals which received the lethal or a higher dose of sarin but not MCC® died within 20 minutes. They showed tremors and convulsions immediately after sarin was applied onto the skin.

Table 2 shows the volumes of sarin solution applied on the mouse skin and detailed data on animals that survived 24 h after the experiment. Without decontamination, sarin caused 50 % (2/4) and 100 % (4/4) mortality in the doses of 1452.86 μg kg^{-1} and 1830.60 μg kg^{-1}, respectively (Figure 1). To evaluate the efficacy of MCC® we used the same two doses of sarin and four higher doses on a second group of NOD mice. Figure 1 shows that MCC® can increase the survival of contaminated mice even at these higher doses. Mice decontaminated with MCC® tolerated the dose of 3662.68 μg kg^{-1}, which is three times the sarin's percutaneous LD_{50} (Table 2). The recovered animals had no skin changes, which was confirmed 24 hours after exposure and treatment.

Together with our earlier findings (13), these results suggest that MCC® is a promising antidote against sulphur mustard and sarin because of a great absorption potential (5, 24-27) owed to its three-dimensional net structure and micro-size inorganic particles.

CONCLUSION

Although sulphur mustard and sarin have a different molecular structure, MCC® was able to

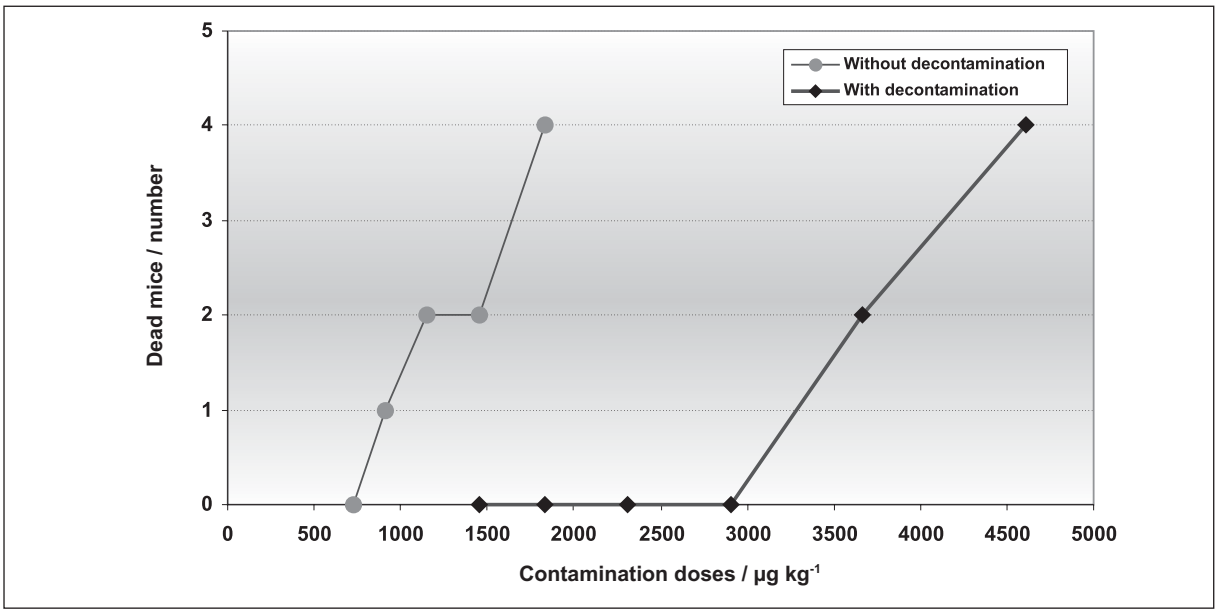

Figure 1 *Mortality of mice (NOD strain) contaminated with LD_{50} sarin solution in isopropanole (c=100 mg L^{-1}) with and without skin decontamination with MCC®*

Table 1 *Skin decontamination against nerve agent sarin determined* in vivo.

Group of mice	Skin poisoning with sarin		
	LD_{50} / µg kg^{-1}	95 % L_R / µg kg^{-1}	TDE
Without decontamination	1221.80	956.31 to 1560.99	/
With decontamination	3662.68	3204.79 to 4186.01	3

TDE - therapeutic decontamination effect
95 % L_R - limit of reliability

Table 2 *Survival of mice (NOD strain) contaminated with LD_{50} sarin solution in isopropanole (c=100 mg L^{-1}) with and without skin decontamination with MCC®*

Group of mice	Volume of LD_{50} sarin solution per mouse / mL	D/C
Without decontamination	0.240	0/4
	0.275	1/4
	0.346	2/4
	0.436	2/4
	0.549	4/4
With decontamination	0.436	0/4
	0.549	0/4
	0.692	0/4
	0.872	0/4
	1.100	2/4
	1.380	4/4

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

absorb them, inactivate, and prevent severe poisoning in mice receiving doses as high as 8.4 and three times the respective percutaneous LD_{50}.

Our finding speaks in favour of current research of inorganic microparticles like MCC® as reactive sorbents for the decontamination of chemical warfare agents (20, 28, 29). However, further *in vivo* studies should elucidate whether the preparation is safe for extensive use in military personnel and whether it is environmentally friendly.

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Sažetak

KOŽNA DEKONTAMINACIJA ŽIVČANOGA BOJNOG OTROVA SARINA S APSORPCIJSKIM PRIPRAVKOM U UVJETIMA *IN VIVO*

Istraživački tim NBKO (nuklearno-biološko-kemijske obrane) radi na pronalasku i razvoju pripravka za dekontaminaciju kože od živčanih bojnih otrova. Cilj ovog istraživanja bio je ispitati dekontaminacijska svojstva (adsorpcijska i/ili kemisorpcijska) pripravka MCC® rabeći živčani bojni otrov sarin kao kožni kontaminant u uvjetima *in vivo.* MCC® je sintetski pripravak koji je biokemijski aktivan i ima ionskoizmjenjivačka i adsorpcijska svojstva. Istraživanje u uvjetima *in vivo* napravljeno je na miševima aplikacijom rastućih doza sarina na kožu životinje. Pripravak MCC® uporabljen je kao kožni dekontaminant neposredno nakon kožne kontaminacije sarinom. Istraživanja su pokazala da pripravak MCC® posjeduje adsorpcijska svojstva, ujedno važna za dekontaminaciju živčanih bojnih otrova. Eksperimenti u uvjetima *in vivo* na miševima (NOD-soj) pokazali su da se dekontaminacijom pripravkom MCC® može postići terapijski učinak od 3 LD_{50} (perkutano, sarin).

KLJUČNE RIJEČI: *dekontaminacija kože, miš, pripravak Mineral Cationic Carrier (MCC®), sarin*

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