Highly toxic organophosphorus compounds (OPs) were originally developed for warfare or as agricultural pesticides. Today, OPs represent a serious threat to military personnel and civilians. This study investigates the in vivo decontamination of male Wistar rats percutaneously exposed to paraoxon and two potent nerve agents - soman (GD) and VX. Four commercial detergents were tested as decontaminants – Neodekont™, Argos™, Dermogel™, and FloraFree™. Decontamination performed 2 min after exposure resulted in a higher survival rate in comparison with non-decontaminated controls. The decontamination effectiveness was expressed as protective ratio (PR, median lethal dose of agent in decontaminated animals divided by the median lethal dose of agent in untreated animals). The highest decontamination effectiveness was consistently achieved with Argos™ (PR=2.3 to 64.8), followed by Dermogel™ (PR=2.4 to 46.1). Neodekont™ and FloraFree™ provided the lowest decontamination effectiveness, equivalent to distilled water (PR=1.0 to 43.2).

KEY WORDS: Argos™, chemical warfare agent, Dermogel™, FloraFree™, mass decontamination, military toxicology, Neodekont™, protective ratio
were tested and compared for their decontamination effectiveness in rats pre-exposed to topical nerve agents soman and VX, as well as to an active metabolite of the OP pesticide parathion - paraoxon (Figure 1). The protective ratios (PR) of detergents were determined against the PR of distilled water using a standard in vivo decontamination test (6, 9).

Animals
A total of 327 adult male Wistar rats (b.w. from 180 g to 210 g) were obtained from the Velaz corp. (Czech Republic) and placed at an approved animal facility at the Faculty of Military Health Sciences (FMH). The animals were housed in standard boxes in groups of six and received standard food and drinking water ad libitum. The handling and treatment of the experimental animals were done under the supervision of the Ethics Committee of the FMH and in full accordance with laws and regulations.

Methods
The acute percutaneous toxicity of the selected OPs was established using a conventional acute toxicity test (10) prior to skin decontamination. Undiluted toxic agents (soman, paraoxon) or hexane diluted VX were topically administered onto clipped dorsal skin (5 cm x 7 cm) using a pipette. Clipping was performed on the day before the experiment to avoid post-clipping irritation. The animals were placed in a fume hood and restrained on plastic platters during agent application/decontamination. Each experimental group consisted of four to six animals. Four to five different doses of the agent were applied. Decontamination was initiated 2 min after exposure by swabbing the dose site manually by 10 strokes in a head-to-tail direction using a cotton swab (2.5 cm x 2.5 cm) moistened in a 5 % aqueous solution of a single detergent type and finally dried by another swab. The temperature of the decontamination fluid was maintained at 25°C. The control group was decontaminated with distilled water instead of a detergent solution following the same protocol. Blank controls (n=5) were fixed, clipped, and “decontaminated” with distilled water without pre-exposure to toxic agents. The animals that survived for 24 h were euthanized by CO₂.

Data analysis
Acute toxicity was evaluated by assessing the median lethal dose (LD₅₀, mg kg⁻¹) and its 95 % confidence limits, calculated by a probit analysis of deaths occurring within 2 h (soman) and 24 h (VX, paraoxon). The effectiveness of decontamination was expressed as the protective ratio (PR); i.e., the ratio between LD₅₀ in decontaminated animals and LD₅₀ in untreated animals (9). The statistical significance of the difference between decontaminated and non-decontaminated LD₅₀ was assessed on the basis of
RESULTS

The rats percutaneously poisoned by OPs showed dose-dependent signs of intoxication. In soman poisoned groups, the local fasciculation of dorsal muscles started usually several (from 3 min to 7 min) after application and expanded gradually. The animals that received supra-lethal doses of soman (from 10 mg kg\(^{-1}\) to 40 mg kg\(^{-1}\)) suffered from strong central convulsions terminated by respiratory crisis (apnoea) and death. When 30 mg kg\(^{-1}\) was administered, convulsions occurred within (5.3±1.9) min. Mastication, salivation, and piloerection were also observed. Dermal LD\(_{50}\) of soman assessed 2 h after exposure was 9.83 mg kg\(^{-1}\) (from 5.87 mg kg\(^{-1}\) to 13.63 mg kg\(^{-1}\)), and it remained the same after 24 h. Compared to soman, VX-poisoned animals showed signs of a cholinergic crisis later on. Local and central convulsions started (91.8±6.1) min after VX administration at a dose of 0.1 mg kg\(^{-1}\). Hyper-salivation, lacrimation, and pilo-erection also occurred as signs of intoxication. Dermal LD\(_{50}\) of diluted VX observed 24 h after exposure was 0.085 mg kg\(^{-1}\) (from 0.027 mg kg\(^{-1}\) to 0.102 mg kg\(^{-1}\)). In paraoxon-treated animals, the first signs of intoxication were apparent approx. 4 h after application (local fasciculation) with 24 h LD\(_{50}\) of 4.37 mg kg\(^{-1}\) (from 1.59 mg kg\(^{-1}\) to 8.84 mg kg\(^{-1}\)). There were no cholinergic signs or deaths within 2 h and 24 h in blank controls.

Animals subjected to decontamination suffered less from cholinergic symptoms compared to non-treated. In all cases, the survival of decontaminated animals increased and deaths were usually related to higher agent doses. In general, the most promising decontamination effectiveness was achieved with Argos\(^{TM}\), followed by Dermogel\(^{TM}\). Lower decontamination effectiveness was found with FloraFree\(^{TM}\) and Neodekont\(^{TM}\) solutions (Table 1, 2, and 3).

DISCUSSION

Acute toxicity of OPs

The toxic effect of OPs is caused mainly by the irreversible inhibition of the enzyme acetylcholineseterase (AChE, EC 3.1.1.7), which leads to the accumulation of the neurotransmitter acetylcholine in the central and peripheral nervous systems and ultimately to a fatal impact on neuromuscular transmission (2, 11, 12). Thus, typical signs of cholinergic crisis were observed in percutaneously intoxicated rats (fasciculation, salivation, etc.). In this study, VX was the most toxic

<table>
<thead>
<tr>
<th>Decontamination effectiveness</th>
<th>Decontaminant vs. soman</th>
<th>Acute toxicity of soman</th>
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LD\(_{50}\) / mg kg\(^{-1}\): 35.85, 30.70, 30.70, 25.83, 26.72, 9.83
LCL / %: 5.98, 5.87
UCL / %: 38.50, 13.63
PR: 3.6, 3.1, 3.1
D – dose in mg kg\(^{-1}\)
Ar - Argos\(^{TM}\); De - Dermogel\(^{TM}\); Fl - FloraFree\(^{TM}\); Ne - Neodekont\(^{TM}\)
LCL - lower confidence limit; UCL - upper confidence limit
Significant difference (p<0.05) between decontaminated LD\(_{50}\) and non-treated LD\(_{50}\) is marked with an asterisk.

The effectiveness is shown as protective ratio (PR; median lethal dose in decontaminated animals divided by median lethal dose in untreated animals). Mortality (M) is shown as the number of deaths versus the number of animals in the experimental group.
agent with LD50 being less than 0.1 mg kg\(^{-1}\), which is in concordance with established data on VX (2), even though it was diluted. In some studies, dilution was found to elevate skin permeation in vitro (13, 14), which could possibly lead to an earlier manifestation of cholinergic signs and higher VX toxicity in vivo (25). On the other hand, Hamilton et al. (15) found little or no effect of dilution on VX absorption. The extreme toxicity of agent VX is caused by a preferential reaction with AChE, contrary to G-agents which are more willing to be hydrolyzed by other ChE (2).

Although VX was the most toxic, cholinergic signs occurred relatively late (1.5 h) compared to soman. When high doses of soman were applied, a rapid manifestation of AChE inhibition occurred in several minutes (fasciculation, central convulsions). In supralethal doses, other cholinergic signs such as hypersalivation or mastication usually did not firmly manifest because of an early onset of central symptoms followed by rapid death. Muscarinic signs were observed mainly in sub-lethal (mainly miosis, hypersalivation, and piloerection) and lethal doses (all of the mentioned signs). The rapidity of soman’s effect can be explained by its high lipophilicity, which supports easy passage through the blood-brain barrier (16). Animals surviving 2 h also survived a 24 h exposure in all cases. In paraoxon, mild cholinergic symptoms (fasciculation) started 1.5 h under a high dose (20 mg kg\(^{-1}\)) and several hours after lower doses (from 2 mg kg\(^{-1}\) to 5 mg kg\(^{-1}\)). Finally, even when the onset of signs was mild, the LD50 of paraoxon was approximately 50 % lower than the LD50 of nerve agent soman after 24 h of exposure.

### Decontamination of OP

Wet type decontamination using detergents reduced the symptoms of OP toxicity. Deaths were either fewer or associated with higher doses of OPs.

#### Table 2 Decontamination effectiveness of detergents against VX

<table>
<thead>
<tr>
<th>Decontamination effectiveness</th>
<th>Decontaminant vs. VX</th>
<th>Acute toxicity of VX</th>
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<td>0.079 2/6</td>
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<tr>
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<td>0.159 6/6</td>
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<td>10.0 4/4</td>
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LD50 / mg kg\(^{-1}\) 5.51 3.92 0.846 3.45 3.67 0.085
LCL / % 3.00 - 0.003 2.10 0.82 0.027
UCL / % 17.95 - 1.889 33.32 9.52 0.102
PR 64.8* 46.1 10.0 40.6* 43.2*

See Table 1 for a more detailed explanation.

#### Table 3 Decontamination effectiveness of detergents against paraoxon

<table>
<thead>
<tr>
<th>Decontamination effectiveness</th>
<th>Decontaminant vs. paraoxon</th>
<th>Acute toxicity of paraoxon</th>
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<tr>
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<td>2.0 0/5</td>
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<tr>
<td>20.0 4/4 20.0 4/4 15.0 4/4 15.0 4/4 12.0 4/4 20.0 4/4</td>
<td>2.0 0/5</td>
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LD50 / mg kg\(^{-1}\) 11.34 10.62 9.91 7.07 4.52 4.37
LCL / % - - 2.65 - 0.52 1.59
UCL / % - - 18.16 - 10.52 8.84
PR 2.6 2.4 2.3 1.6 1.0

See Table 1 for a more detailed explanation.
All of the detergents provided a low or modest level of protection, which was attributed to their low detoxification potency (17, 18) and the low solubility of lipophilic OPs in water solutions (2, 17). The lowest decontamination effectiveness was achieved against paraoxon (PR=1.6 to 2.6), followed by soman (PR=2.6 to 3.6). A more effective decontamination was achieved against VX (PR=10.0 to 64.8) which penetrated tissue slowly (15) and was not significantly absorbed during 2 min of exposure. Moreover, a low dose of VX was easily removed by a moistened cotton swab during decontamination. Argos™ and Dermogel™ were more effective in OP decontamination, whereas Neodekont™ and FloraFree™ provided lower decontamination effectiveness corresponding to that of distilled water or even lower. As for VX decontamination, both Neodekont™ and FloraFree™ were less effective than distilled water, suggesting the enhancement of skin permeation after washing with detergent solutions, as demonstrated in vitro (14, 19, 20). Although the chemical composition of the tested detergents was similar, the highest decontamination effectiveness against all of the tested OPs was consistently achieved with Argos™, which was also the most effective in the decontamination of sulphur mustard (9). The slight difference in the chemical composition of detergents apparently went in favour of Argos™, which is a relatively simple mixture of two primary and one secondary surfactant with a minor additive content to reduce the detergency effect of the surfactants (9).

Several more effective reactive and non-reactive decontamination methods or special preventatives as barrier creams were evaluated as countermeasures against CWAs (e.g., 6, 18, 21, 22). Most of them require skilled or prophylactic application and are appropriate only in the military domain. On the other hand, non-reactive detergents and soapy water are useful in mass decontamination facilities because of their availability, low health risk, and low skill requirements. Despite the fact that none of the studied detergents were able to effectively eliminate OPs from the skin, their use in emergencies could delay a fatal impact on victims. They could provide precious time for transport and subsequent medication of victims by pharmaceuticals such as anticholinergics (atropine) and AChE reactivators (23, 24).

Mass casualty decontamination requires a certain amount of time for technical implementation, which usually delays treatment. The response time is an important factor which influences the effectiveness of the decontamination (15, 17, 25, 26). In this study, decontamination was performed 2 min after exposure and the effectiveness would have certainly been decreased had the treatment been delayed. Another in vivo study (27) showed a good effectiveness for detergents used in the delayed decontamination of VX; however, as demonstrated, VX is a relatively slow-acting agent. Thus, further investigation is needed to evaluate the effectiveness of delayed decontamination against more rapid G-agents.

CONCLUSIONS

The harmful effect of OPs could be counteracted prior to systemic absorption using an appropriate decontamination method. In this study, wet type decontamination using four commercial detergents originally developed for cleaning and decontamination purposes were tested against OPs. All of the detergents generally provided modest protection against soman and paraoxon (PR=1.6 to 3.6). Higher protection was achieved against VX (PR=10.0 to 64.8). According to the obtained results, Argos™ (PR=2.3 to 64.8) should be investigated as a possible decontaminant in mass casualty scenarios. Moreover, the search for more effective alternatives should be considered.

Acknowledgements

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Conflict of Interest Declaration

The authors report no conflicts of interest. The authors alone are responsible for the content of the paper.

REFERENCES


Sažetak

PERKUTANA TOKSIČNOST I DEKONTAMINACIJA SOMANA, VX-A I PARAOKSONA U ŠTAKORA DETERDŽENTIMA

Visokotoksični organofosforni spojevi (krat. OP) prvotno su se proizvodili za ratovanje i uporabi u poljoprivredi kao pesticidi. Danas su nepobitno iznimna prijetnja vojnom osoblju i civilima. Ova studija istražuje in vivo dekontaminaciju muških štakora Wistar perkutano izloženih paraoksonu i dvama snažnim nervnim agensima – somanu (GD) i VX-u. Četiri komercijalna deterdženta ispitana su kao sredstva dekontaminacije - Neodekont, Argos, Dermogel i FloraFree. Dekontaminacija dvije minute nakon izlaganja rezultirala je višom stopom preživljavanja u usporedbi s nedekontaminiranom kontrolnom skupinom. Učinkovitost dekontaminacije izražena je kao zaštitni omjer (PR, medijan smrtonosne doze agenta u dekontaminiranih životinja podijeljen s medijanom smrtonosne doze agenta u nedekontaminiranih životinja). Najveća konzistentna učinkovitost postignuta je Argosom (PR=2.3 do 64.8) i Dermogelom (PR=2.4 do 46.1). Deterdženti Neodekont i FloraFree bili su najmanje učinkoviti i imali jednak učinak kao destilirana voda (PR=1.0 do 43.2).

KLJUČNE RIJEČI: Argos, Dermogel, FloraFree kemijski ratni agens, masovna dekontaminacija, Neodekont, vojna toksikologija, zaštitni odnos

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