

## CYCLODEXTRINES AS FUNCTIONAL AGENTS FOR DECONTAMINATION OF THE SKIN CONTAMINATED BY NERVE AGENTS

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**Summary:** Three decontamination solutions of  $\beta$ -cyclodextrines were prepared. Their abilities to decontaminate rat skin contaminated with nerve agent soman were tested. Decontamination efficacy of the tested cyclodextrine solutions was compared with the same decontamination means but without the cyclodextrines. The efficacy of tested decontaminants was evaluated by the assessment of the ID<sub>50</sub> values. Two decontamination prescriptions with cyclodextrines (tetraborate buffer and tetraborate buffer with acetone) do not show significantly better decontamination efficacies in comparison with prescriptions without cyclodextrines. Only in case of aqueous solution of 2-aminoethanol the addition of  $\beta$ -cyclodextrine resulted in significant increase (32%) in decontamination efficacy.

**Key words:** Cyclodextrine; Nerve agents; Decontamination; In vivo; Soman; Skin; Acetylcholinesterase

### Introduction

Cyclodextrines [CDs] are cyclic oligosaccharides formed by a number of glucose units linked with  $\alpha$ -(1-4) glycosidic bonds. There are three known basic structures that differ only in the number of units. The  $\alpha$ -cyclodextrine [ $\alpha$ -CD], of six units,  $\beta$ -cyclodextrine [ $\beta$ -CD] of seven units and the  $\gamma$ -cyclodextrine [ $\gamma$ -CD]. All of them have as a common feature the existence of a hydrophobic central cavity surrounded by a hydrophilic ring formed by hydroxyl groups (7).

These seminatural artificial receptors can bind a variety of organic, inorganic and biological guest molecules inside their apolar cavities in aqueous solution to form host-guest complexes or supramolecular systems (17).

Therefore, this fascinating property enables them to be successfully used as drug carriers (10,16), separation reagents (6,25), enzyme mimics (2,18) and catalysts of the chemical reactions (20). Relatively little of this work has been concerned with the hydrolysis of the organophosphorus compounds (1,3).

Organophosphorus compounds used at the present time as pesticides [paraoxon] or as chemical warfare agents [sarin, soman, cyclosarin, tabun] belong among irreversible inhibitors of the enzyme acetylcholinesterase [AChE; EC 3.1.1.7] (13,15). AChE plays a key role in the physiological function of the cholinergic nervous system and, therefore, its inhibition is life-endangering (14).

The relatively unsatisfactory antidotal treatment available for acute poisonings with organophosphates has

prompted the study of prophylaxis possibilities that allow survival of organisms exposed to organophosphates (4).

There is considerable interest in methods for the decontamination of the skin after contamination with these agents (8,11). Several methods of nerve agents decontamination are based on the sorption into the porous materials [currently used decontamination mean in the Czech Army, called DESPRACH] (21), on the micellar catalysis (5,12) or on the cyclodextrine host-guest interactions (3,22), as mentioned above.

In this study, we have prepared decontamination solutions of  $\beta$ -cyclodextrines and tested their ability to decontaminate rat skin contaminated with nerve agent soman. Decontamination efficacy of the tested cyclodextrine solutions was compared with the same decontamination means but without the cyclodextrines.

### Material and Methods

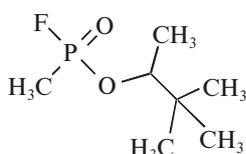
#### Chemicals

$\beta$ -Cyclodextrine [ $\beta$ -CD] was obtained from the Military Technical Institute [VU 070, Brno, Czech Republic]. Solubility in the water solutions, purity and chemical identity of the CD were checked as follows. Solubility in the water solutions was checked at the temperature 25 °C in distilled water. Determined range of solubility agrees with the data described earlier by Steitlim (19) – for  $\beta$ -CD 1,8 g / 100 ml. Solubilities in the solution of sodium hydroxide [pH = 9] and in the solution of sodium hydroxide [pH = 9] mixed with 0,1 M KCl was tested too. Observed range of

solubility does not differ from the values measured for distilled water.

Purity and chemical identity of the CD was tested using thin layer chromatography on the Merck glass plates [*n*BuOH: EtOH: H<sub>2</sub>O - 4:3:3] and using IR spectroscopy [specromether Philips PU 9706]. Other impurities were not observed in the sample. Organophosphate soman [Fig. 1] [O-pinacolylmethylfluorophosphonate] was obtained from the Military facility [VOZ 072, Zemianské Kostolany, Slovak Republic] in 95% purity.

All other chemicals used in this work were commercial products of Merck or Sigma-Aldrich.



**Fig. 1:** Chemical structure of the soman.

**Tab. 1:** Composition of the tested decontamination solutions.

Number	Name of the decontamination solution	Composition*
1	Tetraborate buffer pH =9	32.4 ml of 0.13 M natrium tetraborate 16.7 ml of 0.10 M HCl 50.9 ml distilled water
2	Tetraborate buffer with the addition of the β-CD	32.4 ml of 0.13 M natrium tetraborate 16.7 ml of 0.10 M HCl 50.9 ml distilled water 1.70 g β-CD
3	Tetraborate buffer with acetone	32.4 ml of 0.13 M natrium tetraborate 16.7 ml of 0.10 M HCl 23.1 ml distilled water 27.8 ml acetone
4	Tetraborate buffer and acetone with the addition of the β-CD	32.4 ml of 0.13 M natrium tetraborate 16.7 ml of 0.10 M HCl 23.1 ml distilled water 27.8 ml acetone 1.70 g β-CD
5	water solution of 2-aminoethanol	30 ml 2-aminoethanol 70 ml distilled water
6	water solution of 2-aminoethanol with the addition of the β-CD	30 ml 2-aminoethanol 70 ml distilled water 1.70 g β-CD

\*composition for 100 ml of the final solution

#### Animals

Male white albino Wistar rats weighing 180–200 g were purchased from Velaz Praha [Czech Republic]. They were

kept in an air-conditioned room [22 ± 1 °C and 50 ± 10 % relative humidity] with lights from 07.00 to 19.00 and were allowed free access to standard food and tap water *ad libitum*. The rats were divided into groups of five animals each [n = 5]. Before the experiment they were shaved on their dorsal part [3 x 5 cm]. Experiments were performed under the supervision of the Ethics Committee of Medical Faculty of Charles University and Purkyně Military Medical Academy in Hradec Králové, Czech Republic.

#### Decontamination solutions

There were prepared six kinds of decontamination solutions - tetraborate buffer pH =9, tetraborate buffer with the addition of the β-CD, tetraborate buffer with acetone, tetraborate buffer with acetone and with the addition of the β-CD, water solution of 2-aminoethanol and finally water solution of 2-aminoethanol with the addition of the β-CD. Composition of all tested decontamination solutions is described in the Table 1.

#### In vivo experiments

The efficacy of the cyclodextrine solutions to decontaminate soman contaminated rat skin was determined using modified in vivo decontamination test (4). To determine the decontaminating efficacy of the tested solutions, the rats were poisoned percutaneously [p.c.] with the appropriate dose of organophosphate soman and then they were decontaminated using CDs solutions. The control group were rats contaminated with soman, but decontamination was not performed. The decontamination with CD solutions was started 2 min following p.c. poisoning. The decontamination solution was used at a dose of 1.6 ml per animal and it was spread on the contaminated skin area using defined tampons [weight 327 ± 23.5 mg] within 30 s.

#### Data analysis

The LD<sub>50</sub> values and their 95% confidence limits were calculated by probit analysis of deaths occurring within 24 hours after p.c. administration of soman at five different doses with five animals per dose (23). Efficacies of decontamination solutions were compared using ID<sub>50</sub> values [Protective ratio]. Index ID<sub>50</sub> was calculated from the values of LD<sub>50</sub> measured with the decontamination, and LD<sub>50</sub> without the decontamination.

$$ID_{50} = LD_{50 \text{ p.c. decontaminated}} / LD_{50 \text{ p.c. non-decontaminated}}$$

Just as the index ID<sub>50</sub> is high, the decontamination solution is potent.

## Results

Contamination of the skin of experimental animals with soman was performed as described above. The decontamination action with one of different six decontamination prescriptions tested in our study, was started 2 minutes

after application of toxic organophosphorus compound. The efficacy of tested decontaminants was evaluated by the assessment of the ID<sub>50</sub> values using Weil's test (23). Results for all decontamination receptures are presented in Table 2.

**Tab. 2:** Decontamination efficacy of solutions.

Number	Name of the decontamination solution	ID <sub>50</sub> p.c. (rat)
1	Tetraborate buffer pH =9	6.1
2	Tetraborate buffer with β-CD	5.9
3	Tetraborate buffer with acetone	6.7
4	Tetraborate buffer with acetone and β-CD	7.4
5	Aqueous solution of 2-aminoethanol	12.6
6	Aqueous solution of 2-aminoethanol with β-CD	16.7

All the decontaminants tested show the value of ID<sub>50</sub> higher than 1. Whence it follows that all the prescriptions for decontamination solutions effect as decontaminants of the skin contaminated with soman. When solutions 1 and 2 are compared, it is clear that the decontamination efficacy is decreased by CD addition. On the contrary, in case of tetraborate buffer with acetone [3, 4], the addition of CD resulted in ID<sub>50</sub> increase. Acetone addition into the decontamination solution only slightly increases the ID<sub>50</sub> value. Nevertheless the substitution of tetraborate buffers [solutions 1-4] for 2-aminoethanol solution [solutions 5, 6] significantly enhances the decontamination efficacy of the appropriate solutions. Even in case of solution 6, the value of decontamination efficacy ID<sub>50</sub> achieves 16.7. From results presented in Table 2 it can be concluded that the most effective decontaminant is the prescription consisting of aqueous solution of 2-aminoethanol and β-CD [6].

## Discussion

Well-timed skin decontamination is the crucial step after percutaneous poisoning with chemical warfare agents (4). Several modalities of skin decontamination can be recently applied as described above (5,12,21,22). Cyclodextrines can serve as suitable decontaminants after skin contamination with nerve agents due to their ability to form host-guest complexes (17).

Our in vivo results show that addition of β-CD into the solution of tetraborate buffer and tetraborate buffer with acetone does not cause significant increase in decontamination efficacy. Only in case of aqueous solution of 2-aminoethanol, the addition of β-CD resulted in significant increase [32%] in decontamination efficacy. Ineffectiveness of tetraborate decontaminants has two reasons. At first, neat soman applied on the skin has considerably higher affinity to mixture of lipophile and hydrophile medium represented by skin than to aqueous medium of decontaminating solution (24). In consequence soman does not reach

the CD molecules in short time and sufficient amount and thus the process of decontamination does not run at desirable rate. Slow diffusion from the solid surface into the solution is the direct action of the whole process, instead of the rapid chemical reaction. Secondly, during our experiment it was found that the animal skin is within 1 to 2 minutes after decontamination action completely dry and thus the decontaminating reaction is stopped, because the suitable medium for reaction running is not available and soman with respect to its low volatility proceeds further with penetrating into the skin (24).

The addition of acetone into the decontaminant solution should avoid problems with soman insolubility but it resulted only in slight increase in ID<sub>50</sub> value. Rapid evaporation of solvents from the surface, even potentiated by acetone adding, probably caused lower efficacy than we expected. Therefore acetone was replaced by 2-aminoethanol with much lower volatility in comparison with acetone (9). In addition with regard to 2-aminoethanol, structure there is no presumption of competition with soman for CD cavity occupation.

In conclusion, decontamination prescriptions with CD [2, 4] do not show significantly better decontamination efficacies in comparison with prescriptions without CD. In case of decontaminants with 2-aminoethanol [6], the satisfactory decontamination efficacies were achieved.

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