

## ORIGINAL ARTICLE

# TRANSDERMAL PENETRATION OF THE ACETYLCHOLINESTERASE REACTIVATOR HI-6 IN A RAT MODEL

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### Summary

The objective of the experiment was to verify that HI-6 dimethanesulphonate (HI-6 DMS) is able to penetrate the skin in amounts sufficient to protect against organophosphate poisoning using a rat model. HI-6 2Cl is a major component of Transant, a transdermal patch, used as a protective agent against organophosphate intoxication in the Czech and Slovak armies, although there is little evidence that HI-6 would penetrate the skin in sufficient amounts. HI-6 DMS at a total amount of 127 mg or 635 mg was applied as a buffer solution on the Transant patch which was fixed on the back of the rat. Two, seven or twenty-four hours later, rats were sacrificed and blood samples were collected to determine the levels of HI-6 in plasma by HPLC on reversed phase with isocratic elution and UV/VIS detection. HI-6 was not detectable in plasma samples of animals exposed to 127 mg of HI-6 DMS. The highest levels of HI-6 ( $20.6 \pm 18.8$  ng/ml) were found in plasma of animals exposed to 635 mg of HI-6 DMS 2 hours after patch application, whereas after 7 or 24 hours the levels were very low. Based on these results, the ability of HI-6 DMS to penetrate the skin is discussed and some possibilities of improving the transdermal penetration are suggested.

*Key words: Acetylcholinesterase inhibition; acetylcholinesterase reactivation; organophosphates; nerve agents; HI-6; transdermal penetration; rat model*

## INTRODUCTION

The objective of the experiment was to verify in a rat model that HI-6 dimethanesulphonate

(HI-6 DMS), an oxime used as an antidote against organophosphate poisoning, is able to penetrate skin in amounts sufficient for protection. Organophosphates (OP) are extremely toxic compounds used as pesticides or as nerve agents. The risk for them to be used as chemical warfare agents or during terrorists' attacks is still an actual problem, which requires searching for new and better methods of therapy and especially prevention of OP poisoning [1]. Toxic effects of OPs are based on irreversible acetylcholinesterase (AChE) inhibition by phosphorylating the serine residue in the catalytic

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domain of the enzyme. The therapy of the OP intoxication consists of the administration of three types of drugs, i.e. the muscarinic antagonist atropine, benzodiazepines as anticonvulsants and oximes. Oximes reactivate the OP-inhibited AChE through a nucleophilic attack on the esteric bond, thereby removing the phosphorous conjugate [2]. The reactivation efficiency depends on the chemical structure of the oxime as well as on that of the OP [3]. It means that there is no universal oxime reactivator. Different oximes vary in their effect against different OPs [1,4].

HI-6, on which we focused in the present study, is one of the most efficient reactivators for quite a broad spectrum of OPs. HI-6 is able to reactivate human AChE inhibited by sarin, cyclosarin, VX, Russian V-agent and soman [5]. Especially, treating soman intoxication is quite difficult and other oximes, including pralidoxime and obidoxime, appeared not to be effective enough in the therapy of soman poisoning [6-9]. Although HI-6 has some beneficial effect in the treatment of poisoning by soman, it is only a weak reactivator of human AChE inhibited by tabun [10].

Two salts of HI-6 are currently available. HI-6 2Cl has been studied for many years and it is used in commercially available autoinjectors for self first aid in case of intoxication with OP compounds. Since the dose of HI-6 in the autoinjectors is limited by the low solubility of HI-6 2Cl in water, the more soluble HI-6 DMS was developed [7,11]. No significant differences in the reactivation efficacy of HI-6 2Cl and HI-6 DMS were observed in the guinea pig model [7,9] and no significant differences were observed in the pharmacokinetics in the swine model [7]. Therefore, HI-6 DMS seems to be a promising alternative for HI-6 2CL.

Oximes are first of all intended for the first aid and treatment of OP poisoning, not for prophylactic purposes. In the case of OPs misuse as chemical warfare agents in a military conflict, some prophylactic measures are needed. Thus Transant as a prophylactic agent was introduced into the Czech and Slovak armies. It is a transdermal patch containing 0.8 g of HI-6 2Cl. After its application on a soldier's back, it should provide an 8-hour-lasting protection against nerve agent poisoning [1,12]. Since there is little evidence that HI-6 DMS would penetrate the skin when applied in Transant, this was explicitly investigated in the present study.

## MATERIAL AND METHODS

### *Animals*

Female Wistar rats were purchased from the Velaz Company (Prague, Czech Republic). They were 3 months old with body weight of 180 - 220 g. The animals were kept in the experimental facility (22±2 °C, 50±10% humidity, 12 hours light per day) and food and water was provided *ad libitum*.

The experiment was permitted and supervised by the Ethical Committee of the Faculty of Military Health Sciences, University of Defence, Hradec Kralove, Czech Republic.

### *Animal exposure and sample collection*

The animals were divided into seven groups, N=5 each. Three groups were exposed to 127 mg HI-6 DMS, three other groups to 635 mg HI-6 DMS and one group served as control group exposed only to phosphate-citrate buffer (vehicle). The fur on the rats' back was clipped at the area of 5×5 cm. HI-6 DMS solution in phosphate-citrate buffer (pH 4,4) at the volume of 0.5 ml was applied on a patch from the Transant set. The area of the used patch was adjusted to 5×5 cm. The patch was stuck on the back skin of the rats. To avoid nibbling or licking the patch, rats were fixed to the washer for the first two hours after the patch sticking. The Transant patch is made by an adhesive foil and an absorbent pad. For better fixation on the rats' back and to avoid drying out, it was additionally fixed by an Urgofilm adhesive tape (Urgo Laboratoires, Chenôve, France). After two, seven or twenty-four hours, the patches were removed, the rats were sacrificed by cervical dislocation and blood samples were collected into heparinized tubes immediately.

### *Sample preparation*

Fresh heparinized blood was centrifuged for 10 minutes at 1,000×g and 10 °C. Plasma was separated into a new tube and frozen at -80 °C until assayed. Before the assessment, plasma was deproteinized by adding concentrated TCA in a ratio of 4:1 (plasma : TCA), then centrifuged for 10 minutes at 18,600×g and 10 °C. Using of TCA as a deproteinizing agent was based on previously published papers, where determination of HI-6 or other oxime reactivators by HPLC was described [13,14].

### *HPLC assay*

All analyses were performed on 1260 Infinity series Agilent liquid chromatograph (Palo Alto, CA, USA) composed of degasser, quaternary pump,

light-tight autosampler unit set (50 µl loop), thermostated column compartment and UV/VIS detector. The maximum HI-6 is 310 nm. Agilent ChemStation software (Palo Alto, CA, USA) was used for results analysis.

Analytical column LiChrospher® 60 RP-select B, 250 × 4.6 (5 µm) with installed guard column (4 × 4 RP-select B; Merck, Darmstadt, Germany) was used for reversed-phase chromatography. The mobile phase composition was 80:20 (v/v) purified water/acetonitrile; aqueous component contained 3 mM octansulfonic acid and 1 mM tetramethylammonium chloride, pH = 1.75. The flow rate of the mobile phase was 1.4 ml/min. All chromatograms were obtained under 30 °C temperature.

The calibration curve was established using plasma samples spiked with HI-6. To eliminate the impact of the sample pretreatment on the results, the samples used for the calibration curve were processed

in the same way as the exposed animals' plasma samples. A detection limit of the method was 0.0625 µg/ml and the calibration curve was linear with the correlation coefficient  $r=0.9991$  at the range from 0.1250 µg/ml to 4.000 µg/ml. The retention time of HI-6 was  $3,7 \pm 0,1$  min.

#### Statistics

Statistical analysis was performed using a Kruskal-Wallis ANOVA with multiple comparison.

## RESULTS AND DISCUSSION

In plasma samples of animals exposed to 127 mg of HI-6 DMS, undetectable amount of HI-6 was observed 2, 7 and 24 hours after application.

Low concentrations of HI-6 were found in plasma of the animals exposed two hours to 635 mg of HI-6 DMS (Table 1). The plasma concentrations had

**Table 1.** Concentration of HI-6 in plasma [ng/ml] 2, 7 and 24 hours after exposition to 635 mg of HI-6 DMS. Results are below limit of detection of the used methods and they are not significantly different ( $p<0.05$ ) from the control group and differences between individual groups are not significant, too.

Time of exposure	2 hours	7 hours	24 hours
Plasma concentration of HI-6 [ng/ml] of 5 rats	47.2	0	7.01
	27.1	1.42	0
	23.6	0	2.88
	5.33	0	5.41
	0	13.1	0
Mean [ng/ml]	20.6	2.91	3.06
Standard deviation	18.8	5.75	3.16

decreased to very low levels after 7 and 24 hours. The amount of HI-6 was below the detection limit counted from the calibration curve, but the peaks were quite distinct. For illustration, we present obtained results in table 1, but it is important to remark that our method is not sensitive enough for accurate measuring of such low concentrations. This fact could explain high standard deviations. Additionally, results measured after two hours from the exposure do not differ from other groups including the control group in a statistically significant way.

The dose of HI-6 proposed for a human, used from one autoinjector containing 500 mg of

HI-6 2Cl, produced peak plasma levels of 15 µg/ml in a man [15]. Plasma levels of HI-6 even as high as 170–180 µg/ml have been reported to produce protection against supra lethal doses of soman [16]. For this reason, HI-6 in levels of about 0.0625 µg/ml (the detection limit of the method) are expected not to be sufficient for protection against OPs and measured plasma levels were even lower.

The results do not indicate good transdermal penetration of HI-6 DMS. Though rat skin is not fully comparable to that of human we do not expect that HI-6 DMS for transdermal penetration would be suitable to protect against OP intoxication. Therefore,

we cannot conclude, that the use of HI-6 DMS would improve the effectiveness of Transant.

It is well known that drugs ideally penetrating the skin should have molecular weight of less than 500 Da and relatively high lipid solubility [17]. HI-6 DMS with its molecular weight of 478 Da just meets the first condition, but the molecule is charged and has therefore relatively low solubility in lipids. This also holds for HI-6 2Cl. These findings elicit questions about the ability of HI-6 in such formulations to penetrate the skin and to be protective against OPs.

A possible way to improve skin penetration of a drug is to use a suitable chemical enhancer [17]. Developments of such enhancers for HI-6 are not known from the literature. Another solution for the limited skin penetration of HI-6 is synthesis of a new molecule based on HI-6 with an incorporated part increasing its penetration and keeping its efficacy. In conclusion, new scientific developments are needed to improve the protection of soldiers against OPs.

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