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Comparison of the therapeutic effects and pharmacokinetics of HI-6, HLö-7, HGG-12, HGG-42 and obidoxime following non-reactivatable acetylcholinesterase inhibition in rats

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Abstract. The oximes HI-6, HLö-7, HGG-12, HGG-42 and obidoxime were used in a previously developed rat model to evaluate the therapeutic effects of oximes other than acetylcholinesterase (AChE) reactivation (so-called "non-reactivating effects"). To test this anaesthetized, atropinized and artificially ventilated rats (n = 8 or 16) were poisoned with a three times LD_{50} dose of the potent AChE-inhibitor crotylsarin (CRS, i.v.). CRS-inhibited rat AChE dealkylates instantaneously, thereby excluding AChE reactivation by the oximes. Five minutes after poisoning the rats were treated (i.v.) with an oxime or saline and 10 min later artificial ventilation was terminated. Survival times were determined. Saline-treated animals died within 15 min. In comparison, treatment with HI-6, HLö-7, HGG-12, HGG-42 or obidoxime resulted in a significant prolongation of survival time. In the groups treated with HLö-7, HI-6 or HGG-12, 12–37% of the animals survived more than 24 h. It was investigated whether differences in therapeutic effectiveness are caused by differences in pharmacokinetics of the oximes. The plasma half-lives of HI-6, HLö-7, HGG-12, HGG-42 and obidoxime amounted to 67, 63, 27, 55 and 179 min, respectively. At doses of 75 or 150 μmol/kg, all oximes could be detected in brain and medulla oblongata in similar amounts (6–10 nmol/g tissue). In vitro, all oximes were effective in restoring failure of neuromuscular transmission (NMT) caused by CRS, albeit with varying potency. All oximes bound with affinities in the micromolar range to rat brain muscarinic receptors. The present results show that (1) prolongation of survival time following lethal intoxication with an organophosphate can be achieved by non-reactivating properties of the oximes and (2) the observed differences in a) pharmacokinetics, b) potency to restore NMT and c) affinity for muscarinic receptors of the various oximes do not correlate with the observed differences in therapeutic effectiveness. Therefore, it is concluded that the prolongation of survival must be due to as yet undefined effects in the brain.

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Key words: Acetylcholinesterase – Organophosphate poisoning – Non-reactivating effects – Pharmacokinetics – Survival – Oximes – HI-6 – HLö-7 – HGG-12 – HGG-42 – Obidoxime – Crotylsarin

Introduction

Bispyridinium oximes are known reactivators of organophosphate (OP)-inhibited acetylcholinesterase (AChE) and have been used effectively against OP-induced toxicity in several species, usually in combination with atropine. However, when primate or human AChE is inhibited by the OP soman, rapid dealkylation ("aging") takes place, resulting in a non-reactivatable enzyme (Loomis and Salafsky 1963; Heilbronn and Tolagen 1965; Wolthuis et al. 1981). For this reason oxime therapy has been considered to be of limited value against soman in man. However, HI-6 treatment has been shown to be effective against lethal intoxication in primates, notwithstanding the persistent presence of very low levels of AChE activity in blood and target tissues (Lipp and Dola 1980; Hamilton and Lundy 1989; Van Helden et al. 1992). Therefore, it was suggested that pharmacological effects of HI-6, unrelated to AChE reactivation (henceforth called "non-reactivating effects") could be important in the treatment of OP poisoning.

Various non-reactivating effects of oximes have been described, such as actions on nicotinic and muscarinic acetylcholine (ACh) receptors (Lundy and Tremblay 1979; Amitai et al. 1980; Clement 1981; Kirsch and Weger 1981; Kloog et al. 1985; Valdes et al. 1985) and effects on ion channels associated with the nicotinic ACh-receptor (Alkondon et al. 1988; Tattersall 1993). However, most of these data were obtained in vitro, making extrapolation to the in vivo situation speculative, and the therapeutic relevance of these effects has still to be established.

To be able to investigate the therapeutic potency of these non-reactivating effects and their underlying mechanisms,

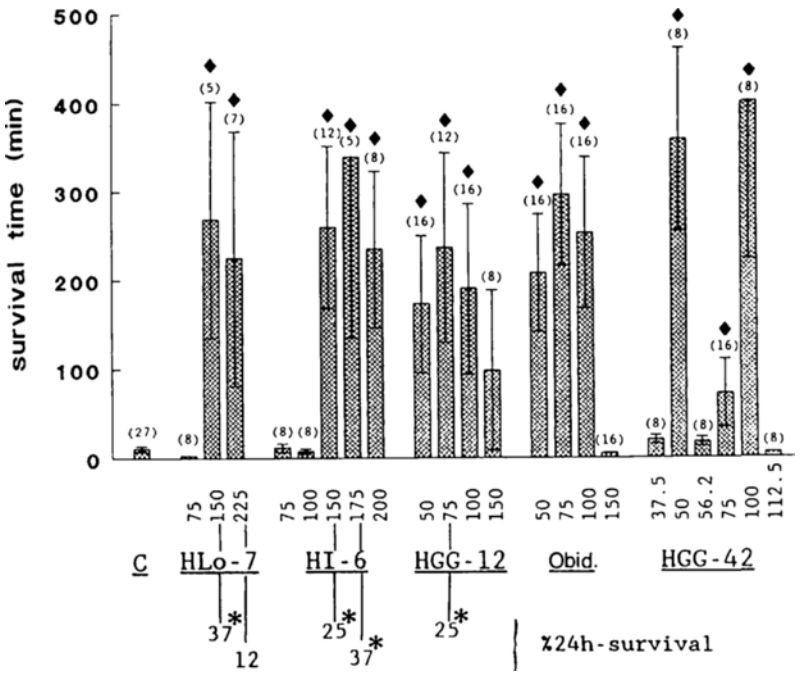


Fig. 1. Survival times (mean ± SEM) of non-24-h survivors (*bars*) and percent 24-h survival (indicated at the bottom) of anaesthetized and atropinized rats poisoned with 3×LD_{50} crotylsarin (i.v.) and treated 5 min later with oxime or saline (control, *C*). All animals were mechanically ventilated starting just before poisoning until 15 min thereafter. The total number of non-24-h surviving animals per oxime dose is indicated at the top and the dose (μmol/kg i.v.) at the bottom of each bar. A group of 8 or 16 rats was used per oxime dose group; the total number of controls was 27. Treatment with all oximes tested resulted in a prolongation of survival compared with controls. Only treatment with HLö-7 (150, 225 μmol/kg), HI-6 (150, 175 μmol/kg) or HGG-12 (75 μmol/kg) led to 24-h survival. Significant differences from control values are indicated by * $p<0.05$ Exact Fisher test; ◆ $p<0.05$ Mann-Whitney *U* test

we developed animal models in which enzyme reactivation could be excluded (Busker et al. 1991; Van Helden et al. 1991). In the model developed by Busker et al. (1991) rats were lethally poisoned with the organophosphate crotylsarin (CRS). In the rat, CRS-inhibited AChE ages instantaneously, resulting in a non-reactivatable enzyme. Like soman, CRS enters the brain very quickly. If treated with HI-6 5 min after poisoning with CRS, 50% of the animals survived, notwithstanding the absence of any AChE reactivation in blood, diaphragm or brain. Hence the therapeutic effects of HI-6 are due to non-reactivating effects. Using this particular model this study was performed with two aims: (1) to compare the non-reactivating therapeutic efficacy of a number of well-known oximes (HI-6, HLö-7, HGG-12, HGG-42 and obidoxime); (2) to assess whether possible differences in efficacy can be ascribed to differences in pharmacokinetics of the oximes and/or to differences in their in vitro potency to cause recovery of CRS-inhibited neuromuscular transmission or to bind to muscarinic receptors.

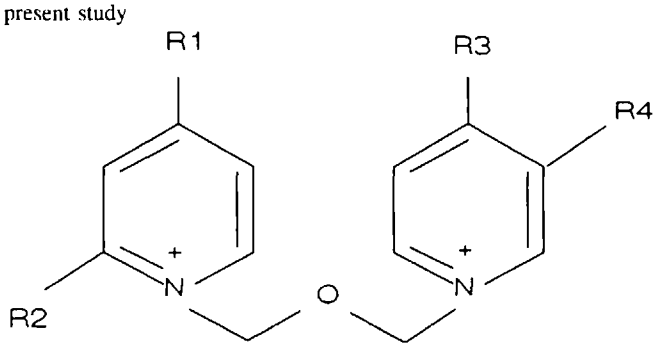
Materials and methods

Survival. Male rats (Small WAG/Rij and WAG/MBL, 180–200 g) were anaesthetized with sodium hexobarbital (Evipan, 175 mg/kg i.p.) and sodium pentobarbital (Veronal, 216 mg/kg i.p.), subsequently atropinized (50 mg/kg i.p.), intubated and injected i.v. with 3 ×LD_{50} CRS 10 min later. Following CRS administration the intubated animals were artificially ventilated for 15 min. Ten minutes before terminating ventilation and removal of the intubation tube, they were treated (i.v.) with an oxime or saline. CRS and the oximes were dissolved in distilled water. At each oxime dose 8 or 16 animals were used (Fig. 1). Survival times of the animals were assessed by keeping them one to a cage and recording (breathing) movements using an ultrasonic detection device.

Blood acetylcholinesterase activity. To determine the effect of the i.v. oxime injection on the course of blood AChE activity after CRS intoxication, rats were treated according to the survival protocol, but in addition they were supplied with a (left) carotid cannula and kept under anaesthesia. Blood samples of 5 μl were collected, immediately mixed with 50 μl 1% saponin and frozen in liquid N_2. After appropriate dilution in 50 mM phosphate buffer pH 7.4, samples were assayed for AChE activity using a radiometric method (Johnson and Russell 1975). In this assay the ACh concentration was 12 μM; [^{3}H]-ACh iodide (acetyl-[^{3}H], specific activity 2.7 GBq/mmol from NEN; Dreieich, Germany) was separated from [^{3}H]-acetic acid, and was diluted to a specific activity of 600 MBq/mmol. Ethopropazine (2.5 μM; Sigma, St Louis, Mo., USA) was used as a specific inhibitor of BuChE. Purified electric eel AChE (Sigma, St Louis, Mo., USA) was used as a reference.

Neuromuscular transmission (NMT). Muscle strips of about 4 mm width and about 20 mm long were cut from isolated diaphragms, all obtained from experimentally naive rats. The strips were kept in Krebs-Ringer solution aerated with 95% O_2/5% CO_2 at room temperature and were provided with a ligature at both ends. Subsequently, the preparations were fixed between two platinum electrodes of a field stimulator to be stimulated indirectly. A displacement transducer served to measure the isometric muscle contractions (Wolthuis et al. 1981). Contractions were registered by means of a Hewlett-Packard recorder. The incubations took place at 37° C in glass vials filled with 60 ml Krebs-Ringer (pH 7.2) aerated with 95% O_2/5% CO_2. The test for neuromuscular transmission was as follows: during 0.2 s a diaphragm preparation was indirectly (supramaximally) stimulated at a frequency which induces a tetanic contraction (40 Hz, pulse duration of 3 μs). This was repeated every 10 s (Van Helden et al. 1991). After a control test at t = 0, CRS (2.4 μM) was added to the bath and incubated for 10 min, then the muscle preparation was incubated for 10 min in buffer containing the oxime. During the experiment the preparation was tetanically (40 Hz) stimulated every 10 s. The area under the curve recorded at t = 20 min was calculated in proportion of that measured at t = 0 (control test) and expressed as a % NMT value which is taken as the NMT recovery due to the non-reactivating activity of the oxime. This is valid, since in such preparations no reactivation of CRS-inhibited AChE was detected (Busker et al. 1991; see also below). A 10-min incubation at each concentration of oxime was sufficient to measure a maximal effect. At least four preparations per oxime dose

Table 1. Chemical structures of the oximes under investigation in the present study



	R_1	R_2	R_3	R_4	X
HI-6	H	CH = N-OH	C(=O)-NH_2	H	Cl
HGG-12	H	CH = N-OH	H	C(=O)-C_6H_5	Cl^-
HGG-42	H	CH = N-OH	H	C(=O)-C_6H_{11}	Cl^-
Obi-doxime	CH = N-OH	H	CH = N-OH	H	Cl
HLö-7	CH = N-OH	CH = N-OH	C(=O)-NH_2	H	I

were used. In some cases, parts of the muscle strips were used to determine AChE activity (Busker et al. 1991).

Pharmacokinetics of the oximes used. Rats were treated according to the survival protocol, but in addition they were supplied with a (left) carotid cannula and kept under anaesthesia. Blood samples of 25 µl were taken repeatedly (see Fig. 4) and centrifuged for 10 min to obtain plasma. Trichloroacetic acid (final concentration 0.02 M) was added, and after 15 min (0° C) this was centrifuged for 10 min and 20-µl aliquots of proper dilutions of the supernatant were injected into an HPLC system. For determination of HI-6 and obidoxime the method described by Hamilton and Lundy (1989) was used. The HPLC system comprised a Spectroflow 400 pump (ABI, Maarssen, Netherlands), a Kontron 460 autosampler, an ABI 757 Absorbance meter, set at 304 nm and a LDC Milton Roy (Hasselroth, Germany) CI 10B integrator. A Chromsep C18 100×3.0 mm column (Chrompack, Middelburg, Netherlands) was used. The mobile phase contained 0.1 M KH_2PO_4 (pH 4.8), 300 mg sodium octylsulphonate, 50 mM triethylamine (Aldrich) to reduce tailing and 3% (v/v) acetonitrile per litre water and was pumped at 0.5 ml*min $^{-1}$. HGG-12 and HGG-42 eluted extremely slowly under these conditions; therefore the system was adjusted by omitting sodium octylsulphonate, and increasing acetonitrile to 7% in the case of HGG-12 (retention time 3 min) and to 10% in the case of HGG-42 (retention time 5 min) for the determination of these two oximes. All samples were kept below 10° C prior to injection. Under these conditions, all oximes appeared to be sufficiently stable.

Oxime concentrations in brain (cerebrum) and medulla oblongata were determined in rats sacrificed 5, 15 or 30 min after oxime injection. Brains and medulla were homogenized (1:10) in the mobile phase, 1/10 volume 0.2 M trichloroacetic acid was added and samples were further treated as above, except that 75 µl of undiluted supernatant was injected into the HPLC. A correction was made for the amount of oxime present in brain blood (Ligtenstein and Kossen 1983). In this respect, *blood* oxime concentrations were multiplied by the haemoglobin content in brain or medulla oblongata (Gorter and De Graaf 1956) and this was subtracted from the total contents of oxime in brain or medulla.

Displacement of [^{3}H]-quinuclidinyl benzilate (QNB) binding. Rats were decapitated, and their brains were quickly removed. After excision of cerebellum each brain was homogenized (1:20) in 30 mM HEPES buffer pH 7.4 containing 0.5 mM EGTA. This was centrifuged for 10 min at 1000 *g*, and thereafter the supernatant was centrifuged for 20 min at 48 000 *g*. The resulting pellet was resuspended and the last centrifugation step was repeated. Following resuspending, the protein concentration was adjusted to 2 mg/ml, and these membrane suspensions were kept at -80° C.

The binding assays were performed in 20 mM HEPES. Specific binding was determined with 50 µl [^{3}H]-QNB, 150 µl drug solution 700 µl HEPES and finally 100 µl membrane suspension. After incubation for 1 h at 25° C under shaking, the incubation was terminated by rapid vacuum filtration over Whatman GF/C glass fibre filters using a Millipore (Etten-Leur, Netherlands) sampling manifold. The filters were washed three times under vacuum with 3 ml ice cold buffer. The filters were placed in vials containing 5 ml scintillation cocktail, and counted at least 3 h later. Non-specific binding was defined as residual binding in the presence of 10 µM atropine sulphate. Maximal binding, K_d, K_i and pseudo-Hill coefficients were calculated after fitting the individual curves. Binding characteristics of each oxime were determined in three independent experiments.

Compounds. Crotylsarin (trans-2-butenyl methylphosphonofluoridate) was a gift of Dr. H. P. Benschop of the Prins Maurits Laboratory TNO; it was greater than 98% pure (GLC) and had satisfactory elementary analysis, NMR and IR spectra; it was stored in dry isopropanol at −20° C and diluted with water immediately before use. HGG-12 [1-(3-phenylcarbonylpyridinio)methoxymethyl-2-hydroxyiminomethylpyridinium dichloride monohydrate] and HGG-42 [1-(3-cyclohexyl carbonyl-pyridinio)methoxymethyl-2-hydroxyiminomethylpyridinium dichloride] were synthesized by Dr. H. P. Benschop. HI-6 [1-(4-aminocarbonylpyridinio)methoxymethyl-2-hydroxyiminoethyl-pyridinium dichloride monohydrate] was made available by Dr. J. G. Clement, Defence Research Establishment Suffield, Canada. Obidoxime (Toxogonin® [1,1'-oxybis(methylene)-bis(4-hydroxyiminomethyl pyridinium) dichloride] was donated by Merck, Germany. HLö-7 diiodide [1-(4-aminocarbonylpyridinio)methoxymethyl-2,4-bis(hydroxyiminomethyl-pyridinium diiodide] was donated by Dr. P. Eyer, Walther-Straub-Institut fur Pharmakologie und Toxikologie der Ludwig-Maximilians-Universität München, Germany. The chemical structures of the oximes are shown in Table 1.

Statistics. Differences in the number of 24-h survivors between saline and oxime-treated groups of rats were tested using the Exact Fisher test; differences in ranking of survival times of individuals in control and experimental groups were tested by the Mann-Whitney *U* test.

Results

Survival

Control animals poisoned with 3×LD_{50} CRS (i.v.) and subsequently treated with saline instead of oxime were incapable of spontaneous breathing for more than 15 min when artificial ventilation was terminated 10 min following the saline injection (Fig. 1). Treatment with HLö-7, HI-6, HGG-12, obidoxime or HGG-42 resulted in a significant prolongation of survival time compared with controls. HLö-7 (150 or 225 µmol/kg) resulted in 37% (3/8) and 12% (1/8) 24-h survival, respectively. HI-6 (150 and 175 µmol/kg) treatment resulted in 25% (4/16) and 37% (3/8) 24-h survival, respectively. HGG-12 (75 µmol/kg) treatment led to 25% (4/16) 24-h survival. Treatment with obidoxime or HGG-42 did not result in 24-h survival.

Blood acetylcholinesterase activity

In all CRS-poisoned rats, blood AChE activity dropped to less than 1% of the starting activity within 3 min. Injection

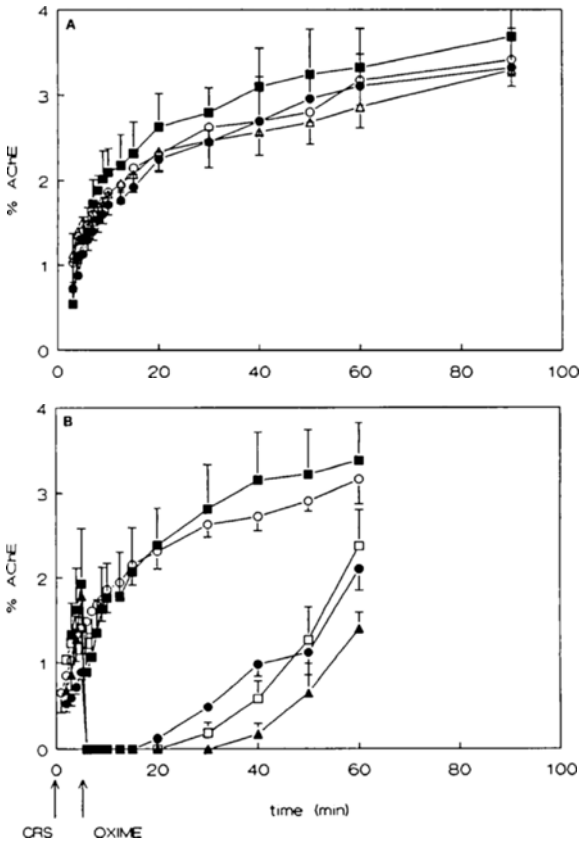


Fig. 2. A Blood AChE activity (mean ± SEM; $n = 4$) in rats poisoned with 3×LD_{50} CRS (i.v. at t = 0) and injected i.v. 5 min later with saline (○), HI-6 (△, 150 µmol/kg), HGG-12 (■, 75 µmol/kg) or HGG-42 (●, 75 µmol/kg). AChE activity is expressed as percent of control value determined just before CRS injection. All animals were artificially ventilated, starting just before poisoning until the end of the experiment. There is no difference between saline- and oxime-treated groups. **B** Blood AChE activity (mean ± SEM; $n = 3$) in rats poisoned with 3×LD_{50} CRS (i.v. at t = 0) and injected i.v. 5 min later with saline (○), HLö-7 (■, 150 µmol/kg), or obidoxime: 37.5 µmol/kg (●), 75 µmol/kg (□) or 150 µmol/kg (▲). In contrast to the other oximes, administration of obidoxime resulted in increased enzyme inhibition

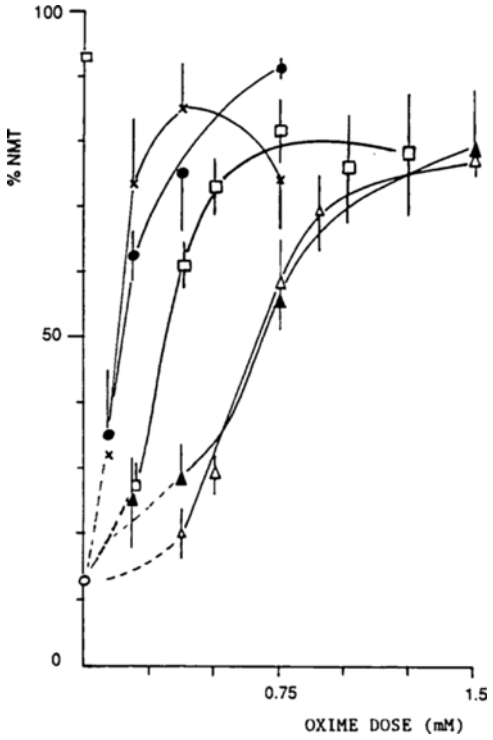


Fig. 3. Dose-response curves of the effects of HI-6 (△), HLö-7 (□), HGG-12 (✖), HGG-42 (●) and obidoxime (▲) on neuromuscular transmission (*NMT*) in rat diaphragm preparations in vitro following complete block by CRS (2.4 µM, ○). The spontaneous NMT decline to about 93% in preparations which were incubated during 20 min in the absence of CRS and oxime is indicated by the *square symbol* at the vertical axis. All values are means ± SEM

of HI-6 (150 µmol/kg), HGG-12 (75 µmol/kg), HGG-42 (75 µmol/kg) and HLö-7 (150 µmol/kg) to rats min after CRS injection had no effect on the course of AChE activity in blood (Fig. 2). In saline- as well as in oxime-treated rats a spontaneous increase of AChE activity took place, amounting to about 2% in the first hour. Treatment with obidoxime, however, resulted in a dramatic fall in AChE activity even below the limit of detection (<0.1% of starting activity, Fig. 2B). Dose-dependently, recovery was detected 10–25 min after obidoxime injection.

Neuromuscular transmission

Tetanic contractions of untreated muscle preparations declined spontaneously by about 7% during the experiment (Fig. 3). Taking this into account, the five oximes tested were all able to restore almost completely the failure of NMT caused by CRS. HGG-12 and HGG-42 were effective at lower concentrations than HI-6 and obidoxime, the ED_{50} (half maximal response) for both HGG oximes was about 0.1 mM and that for HI-6 and obidoxime approximately 0.6 mM. HLö-7 takes an intermediate position (ED_{50} = 0.3 mM). In all cases the recovery of NMT was halted when the oximes were removed from the muscle preparations by washing (not shown). This underlines that the recovery was caused by non-reactivating effects.

The AChE activity in the muscle preparations dropped to 6.4 ± 0.2% of starting activity following 2.4 µM CRS, and was found to be unchanged (6.3 ± 0.1%) 10 min after the subsequent addition of 0.5 mM HI-6 ($n = 4$).

Pharmacokinetics of the oximes used

The course of plasma concentration of the oximes was tested under conditions related as closely as possible to the survival experiments (Fig. 4). The HI-6 dose of 150 µmol/kg was adopted from earlier studies (Busker et al. 1991; Van Helden et al. 1991). HLö-7 and obidoxime, being similarly effective in vitro, were also given at this dose, whereas HGG-12 and HGG-42, the more potent drugs, were given at 75 µmol/kg. For comparison 75 µmol/kg HI-6 was also included. Bi-exponential curves were obtained, and kinetic data were calculated according to two-compartment kinetics (Table 2). Plasma is defined as the

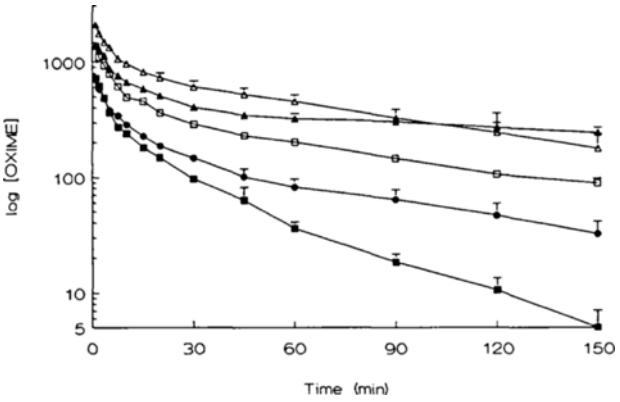


Fig. 4. Time-course of plasma concentrations of HI-6 (△, 150 μmol/kg), HLö-7 (□, 150 μmol/kg), HGG-12 (■, 75 μmol/kg), HGG-42 (●, 75 μmol/kg) and obidoxime (▲, 150 μmol/kg), administered (i.v.) to rats which had been intoxicated with 3 $\times LD_{50}$ CRS 5 min earlier. The rats had been anaesthetized, atropinized and were ventilated just as in the survival experiments. Each point represents means ± SEM of 5–6 animals

central compartment, all other tissues as peripheral compartments. HGG-12 was the fastest eliminated oxime (t½ 27 min). The elimination half-lives of HI-6, HGG-42 and HLö-7 were similar (67, 55, 63 min, respectively). The volumes of the central compartment were 71–107 ml/kg, that of the peripheral compartment about twice this value. Obidoxime was eliminated much more slowly, with an elimination half-life of 179 min.

All oximes entered the central nervous system; the levels were corrected for the amount of blood present in brain and medulla (5 μl/g and 7 μl/g, respectively) (Fig. 5). It appeared that therapeutically effective doses of HI-6, HLö-7, HGG-12 and HGG-42 (150, 150, 75 and 75 μmol/kg, respectively) all resulted in levels of approximately 6–8 nmol/g tissue, both in brain and in the medulla oblongata. Injection of 75 μmol/kg HI-6 led to somewhat lower levels. After injection of 150 μmol/kg obidoxime somewhat higher concentrations were reached. In blood the oxime concentration dropped more quickly during the first 30 min than in brain and medulla. Similar amounts of oximes were found in brains and medulla oblongata from non-poisoned, but otherwise similarly treated animals (results not shown).

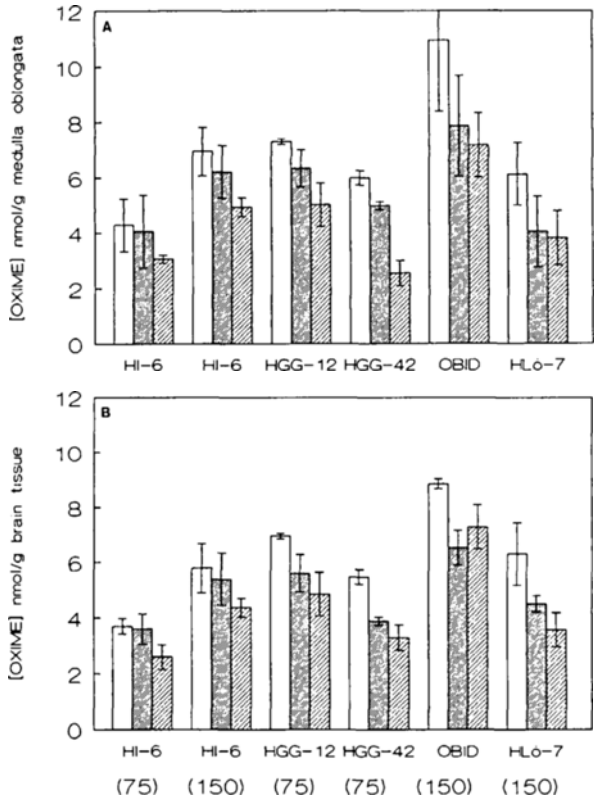


Fig. 5. Amounts of oxime (means ± SEM) detected in medulla oblongata (**A**) and in brain (**B**), corrected for the presence of blood in the tissue. All rats were kept under anaesthesia, they had been intoxicated with $3 \times LD_{50}$ CRS, 5 min prior to oxime treatment (dosed as indicated between brackets, μmol/kg), and they were sacrificed 5 (*open bars*), 15 (*grey bars*) or 30 (*hatched bars*) min after i.v. administration of the oxime

Displacement of [³H]-QNB binding

As can be seen in Table 3, all the oximes under investigation could displace [³H]-QNB binding in rat brain membrane preparations. All inhibition constants are in the micromolar range, with HGG-12 and HGG-42 as the most potent compounds. HI-6 had the lowest affinity. Atropine has a much higher affinity, with a K_i of 1.5 nM.

Table 2. Pharmacokinetic data of oximes administered i.v. to CRS-poisoned rats, calculated according to two-compartment kinetics

Oxime:	HI-6	HI-6	HLö-7	HGG-12	HGG-42	Obidoxime
Dose μmol/kg	75	150	150	75	75	150
n =	6	6	6	6	5	6
α	0.13 ± 0.05	0.19 ± 0.05	0.23 ± 0.01	0.20 ± 0.05	0.15 ± 0.02	0.14 ± 0.03
A	596 ± 142	1290 ± 215	1158 ± 101	553 ± 38	494 ± 23	996 ± 158
β	0.015 ± 0.003	0.010 ± 0.002	0.011 ± 0.001	0.026 ± 0.002	0.013 ± 0.002	0.004 ± 0.001
B	228 ± 40	830 ± 212	427 ± 25	216 ± 19	203 ± 23	458 ± 39
t½ overall (min)	55 ± 7	62 ± 28	63 ± 5	27 ± 3	55 ± 9	180 ± 26
Mean residence time (min)	73 ± 7	88 ± 33	79 ± 7	31 ± 3	69 ± 9	231 ± 34
V_d central compartment (ml/kg)	91 ± 12	70 ± 3	95 ± 11	97 ± 3	107 ± 5	103 ± 6
V_d peripheral compartment (ml/kg)	197 ± 17	148 ± 12	277 ± 22	204 ± 8	258 ± 31	291 ± 18

Table 3. Displacement of [^{3}H]-QNB binding in rat brain membrane preparations; the QNB concentration was 200 pM; $K_d = 223 \pm 19$ pM; $B_{max} = 2.07 \pm 0.07$ pmol/g protein. Hill coefficients were in all cases close to 1

Ligand	K_i (μM) $n = 4$
Atropine	0.0015 ± 0.0003
HI-6	89 ± 23
HLö-7	29 ± 7
HGG-12	2.0 ± 0.3
HGG-42	3.2 ± 0.8
Obidoxime	12 ± 4

Discussion

The therapeutic effectiveness of non-reactivating effects of oximes after intoxication was evaluated. On the basis of the present results the non-reactivating effects of the oximes tested (in combination with atropine) enhance survival after intoxication with CRS in rats. In comparison with controls, survival times of oxime-treated animals significantly increased and treatment with HLö-7, HI-6 or HGG-12 even led to 24-h survival of several animals in the various treatment groups. None of the oximes tested increased blood AChE activity (Fig. 2). Since both the concentrations of the oximes and of the CRS-inhibited AChE, and consequently the likelihood of reactivation, will be greatest in blood, monitoring of the AChE activity in blood can be considered as the most sensitive way to exclude oxime-induced reactivation under the conditions used in the survival protocol (Busker et al. 1991). Thus, it may be concluded that in surviving animals both the failure of neuromuscular transmission in the diaphragm and failure of the neuronal transmission in the respiratory centers caused by CRS have been restored by non-reactivating effects of the oximes. This confirms part of the results of two previous studies, in which soman- (Van Helden et al. 1991) or CRS-poisoned- (Busker et al. 1991) and HI-6-treated rats survived. In both studies any significant reactivation of inhibited AChE measured in blood, diaphragms or in the brain following HI-6 treatment was absent. Earlier experiments in which rats were lethally poisoned with soman and shortly thereafter treated with HI-6 revealed high protective ratios. Survival was mainly attributed to AChE reactivation by HI-6 (Kepner and Wolthuis 1978). In primates, however, rapid aging of soman-inhibited AChE prevents reactivation of AChE. Nevertheless, in experiments with rhesus monkeys (Hamilton and Lundy 1989) and marmosets (Van Helden et al 1992) survival after soman intoxication could be predominantly attributed to non-reactivating effects of HI-6.

Obidoxime significantly prolonged the survival time after CRS; surprisingly, it even caused a decrease in AChE activity. Administration of obidoxime (150 μmol/kg) alone had no effect on AChE activity (results not shown). An explanation for this additional enzyme inhibition by obidoxime might be that this oxime reacts with a metabolite of CRS yielding a new, potent inhibitor. CRS itself is most likely not involved, for it cannot be detected 5 min after i.v. injection (Busker et al. 1991). Reaction of OPs with obidoxime into AChE-inhibiting products has been reported (Alioth-Streichenberg et al. 1991). The fact that administration of obidoxime, while enhancing AChE inhibition, increased survival times of CRS-poisoned animals indicates the importance of the non-reactivating effects of this oxime.

One of the questions to be answered was whether differences in pharmacokinetics of the oximes might explain the difference in their therapeutic effectiveness: treatment with HGG-42 and obidoxime did not yield any 24-h survivors. On the other hand, obidoxime and especially HGG-42 had a strong non-reactivating effect in vitro in restoring NMT after CRS. The kinetic profile of HGG-42 is not much different from that of the more effective oximes, HI-6, HLö-7 and HGG-12. Furthermore, the levels of HGG-42 detected in brain and medulla oblongata after a dose of 75 μmol/kg are similar to those found after therapeutic doses of HI-6, HLö-7 or HGG-12. Obidoxime was eliminated more slowly, and the levels detected in the brain were somewhat higher compared with the levels of HI-6 and HLö-7 after equimolar doses. Apparently, the lack of effectiveness of HGG-42 and obidoxime in 24-h survival is not caused by inadequate pharmacokinetics, but rather by missing efficacy, or by toxicity.

From the relatively short plasma elimination half-lives of the oximes tested (27–67 min) a doubtful therapeutic effectiveness might be expected (Hobbiger 1976). However, as stated above, non-reactivating effects restoring CNS transmission are particularly important for survival. Elimination of HI-6 from the CNS is slower than from plasma (Ligtenstein and Kossen 1983; Ecobichon et al. 1990). Although the small number of observations in the brain does not allow calculation of kinetic parameters, our data show that 21% of HI-6 was eliminated from the brain (mean of both doses tested) between 5 and 30 min following administration, whereas 57% was eliminated from plasma. Similar results were found with HLö-7, HGG-12 and HGG-42. This means that the drugs remain longer in the brain than might be expected on the basis of plasma half-lives. Slow elimination of the oximes from the rat brain tissue could partly explain their effect of prolonging survival time after CRS intoxication.

All five oximes dose-dependently restored CRS-inhibited NMT in the isolated rat diaphragm. However, their potency was not correlated with their effectiveness in prolonging survival. For example, HGG-42 was more potent on NMT than HI-6, whereas HI-6 was more effective on survival. With all oximes tested almost complete recovery of NMT could be achieved. It has been suggested that the skeletal type nicotinic ACh-receptors are affected by oximes (Clement 1981; Su et al. 1983; Broomfield et al. 1987), in particular the receptor ion channel (Alkondon et al. 1988; Tattersall 1993). Currently, we are investigating the contribution of post- as well as presynaptic effects of the oximes on this recovery to unravel the mode of action by which the oximes exert this non-reactivating effect.

All five oximes displaced bound [^{3}H]-QNB from rat brain membrane preparations with K_i values in the micromolar range. The K_i values are in agreement with the data of Kloog et al. (1985), with the possible exception of obidoxime (12 ± 4 μM) which they reported to be 42 ± 6 μM.

Since HI-6 was least effective, whereas HGG-42 and HGG-12 were most effective, any correlation with therapeutic potency is lacking. Since in the present survival experiments the animals were pretreated with atropine (144 μmol/kg), which appears to have a 1000-fold higher affinity for muscarinic receptors than the oximes, muscarinic binding of the oximes cannot play an important role in survival.

HLö-7, HI-6 and HGG-12 were more effective in our survival experiments than obidoxime and HGG-42, indicating that rather small structural differences between the oximes may result in different pharmacological properties. The presence of a phenylcarbonyl moiety (HGG-12) or a cyclohexylcarbonyl moiety (HGG-42), and for obidoxime, HI-6 and HLö-7 the number and position of the oxime moieties (HC = N-OH) or the presence of an aminocarbonyl moiety ($C(O)NH_2$) (see Table 1), may be responsible for these different pharmacological effects.

Until now HI-6 has been regarded as the most promising compound against poisoning by soman (Clement 1981; Wolthuis et al. 1981; Boskovic et al. 1984; Hamilton and Lundy 1989; Van Helden et al. 1992). HLö-7 – tested in mice (Clement et al. 1992; Eyer et al. 1992), guinea pigs (Lundy et al. 1992) and dogs (Eyer et al. 1992) – is presently regarded as the "first broad spectrum oxime *reactivator*" (Clement et al. 1992; Eyer et al. 1992; Lundy et al. 1992). From the present investigation it appears that, also from a "*non-reactivating*" point of view, HLö-7 is at least as effective as HI-6. In conclusion, no consistent correlation exists between the in vivo (survival and pharmacokinetics) and in vitro results (NMT recovery, binding to muscarinic receptors), suggesting that differences in survival may be due to yet unknown effects of the oximes in the brain.

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