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The pharmacokinetics and pharmacodynamics of two HI-6 salts in swine and efficacy in the treatment of GF and soman poisoning

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Abstract

Anesthetized pigs were injected i.m. with 500 mg HI-6 dichloride (HI-6 2Cl) (1-[[[4-(aminocarbonyl)-pyridinio]methoxy] methyl]-2[(hydroxyiminomethyl]pyridinium dichloride; CAS 34433-31-3)) or the molar equivalent of HI-6 dimethanesulphonate (HI-6 DMS) 633 mg. Plasma HI-6 concentrations were measured by HPLC (1, 3, 5, 10, 15, 30, 60 min and every 30 min until 4 h or 6 h following the i.v. or i.m. dose respectively) while a variety of physiological responses were continuously examined. HI-6 (500 mg 2Cl or 633 mg DMS) resulted in an identical pharmacokinetic profile unaffected by atropine co-administration. Neither HI-6 salt resulted in clinically significant changes in cardiovascular or respiratory function. HI-6 DMS (1899 mg i.v.) resulted in plasma HI-6 concentrations about 10 times higher than measured following i.m. 500 mg 2Cl or 633 mg DMS and resulted in small transitory effect on mean arterial pressure. Atropine plus HI-6 DMS (1–9 mg/kg or 127–172 mg/kg i.m.) protected up to 100% of guinea pigs exposed to 5 × LD_{50} of GF (cyclohexyl methyl phosphonoflouridate) or soman (pinacolyl methylphosphonofluoridate) (GD) respectively. The results suggest that the two HI-6 salts have a similar pharmacokinetic profile while HI-6 DMS appears extremely safe and effective against nerve agents and may be as suitable for human use.

Keywords: Organophosphate nerve agents; Oximes (HI-6); Pharmacokinetics; Pharmacodynamics; Protective ratio

1. Introduction

HI-6 2Cl is an oxime that has generated significant interest due to its wide spectrum of therapeutic activity against organophosphate nerve agents (ONA). Compared with traditional oximes such as pralidoxime chloride (2 hydroxy-[iminomethyl]-1- methylpyridinium chloride:2-PAM), it is particularly effective in treating poisoning by agents such as soman (Hamilton and Lundy, 1989; Amitai et al., 1995), GF (Lundy et al., 1992; Clement, 1992; Kassa and Bajgar, 1995) and VR (Maxwell et al., 1997). HI-6 is a good reactiva-

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tor of human erythrocyte acetylcholinesterase inhibited by ONA's that are variably resistant to therapeutic treatment by other licensed oximes such as pralidoxime (2-PAM) and obidoxime chloride (Worek et al., 1998). HI-6 appears from human trials and preclinical toxicity testing, to be safe for human use at the doses which have been examined to date including 500 mg i.m. (Kušic et al., 1985; Jovanovic et al., 1990; Kušic et al., 1991; Clement et al., 1995). This oxime is currently supplied to military personnel in certain countries in wet/dry autoinjectors for first aid use in the event of ONA exposure and if licensed for human use, may be used by first responders in civilian terrorist situations. Each autoinjector contains 500 mg of HI-6 2Cl (and 2 mg of atropine) and military personnel carry three autoinjectors for use as dictated by the severity of the poisoning. The dose of 500 mg/HI-6 was originally chosen for inclusion in the injector principally due to the fact that this dose was near the limits of solubility of HI-6 2Cl in the volume in the fielded device (see Thiermann et al., 1996b) and also because this dose caused no adverse effects in human clinical trials (Kušic et al., 1985, 1991; Jovanovic et al., 1990; Clement et al., 1995). A possible drawback of these autoinjectors is that 500 mg (1500 mg if all three injectors are used) may be a less than an optimal therapeutic dose of HI-6 in a severely poisoned individual. Furthermore, the dose contained in the autoinjector (500 mg HI-6 2Cl) dissolves less rapidly than would be considered desirable under these circumstances. These solubility concerns suggested that in an emergency situation that the needle might become blocked with undissolved crystals preventing the injection of not only HI-6 but also of atropine. These concerns may be overcome following the demonstration that the DMS salt of HI-6 is considerably more soluble than the 2Cl salt especially at low ambient temperatures (Thiermann et al., 1996b). In addition, it appears to have identical cholinesterase reactivating properties to the 2Cl salt in vitro (Krummer et al., 2002) suggesting that the mechanism of action of the two salts is identical. However, in clinical trials to date, neither the optimally protective dose nor the maximally tolerated safe dose of HI-6 has been determined in humans. Therefore, more HI-6 than is currently available in the autoinjectors might prove to be both more efficacious and yet still be safe for use. The use of a larger dose of HI-6 than currently available remains a possibility and could provide significant additional protective effects providing more HI-6 could be dissolved in the autoinjectors following substitution of higher amounts of the DMS salt for the 2Cl salt. However, use of the DMS salt depends on its approval or licensing for human use. In order to obtain a human license, regulatory agencies requires comparative studies on the two salts including the mechanism of action (Krummer et al., 2002) as well as pharmacokinetics/pharmacodynamics and efficacy data. In the present studies, we attempt to establish a close relationship of the two salts with respect to these parameters.

The present studies were undertaken to directly compare the pharmacokinetics of the two salts in an anesthetized pig model. The animals were fully instrumented so that during the pharmacokinetic studies a wide variety of physiological responses could be continuously recorded in order to determine possible differences in physiological responses to HI-6 DMS and HI-6 2Cl. Since it was of interest to gain further information regarding the toxicity of HI-6 DMS and to obtain an estimate of a safe i.v. dose, we also carried out pharmacokinetic and pharmacodynamic evaluations following very large doses of HI-6 DMS injected i.v.

A second part of the study was carried out to examine the efficacy of the new salt against organophosphorus nerve agents which are known to be resistant to the traditional oximes such as 2-PAM or obidoxime (1,3-Bis(4-hydroxyiminomethylpyridinium)-2-oxapropane dibromide). These studies were also carried out to demonstrate the absolute necessity for using sufficient doses of oxime in the treatment of ONA's to ensure maximal protective activity.

2. Methods

2.1. Animals:protection studies in guinea pigs

Male guinea pigs (Hartley strain) 400–500 g (4–5 weeks old) were purchased from Charles River Laboratories (Quebec, Canada) and were used in these studies. Guinea pigs were housed 4/cage with a 12 h light/dark cycle and fed Purina guinea pig chow and allowed water ad libitum. Animals were exposed to nerve agents between 09:00 and 10:00 h each day of the experiment to avoid possible diurnal variation.

2.2. Pharmacokinetic studies in swine

2.2.1. Animals

For the comparison of the pharmacokinetics of the two salts of HI-6 twenty four 9–10 week-old castrated juvenile male Yorkshire–Landrace cross pigs (*Sus scrofa domestica*) weighing approximately 20 kg (20.3 ± 1.93 kg, mean ± S.D.) were obtained from a single local commercial supplier and housed indoors at the vivarium complex at Defence Research Development Canada-Suffield (DRDC Suffield). Animals received hog grower (United Grain Growers, Okotoks, Alberta, Canada) and were allowed tap water ad libitum. An additional four animals were used in studies of the pharmacokinetic/pharmacodynamic profile following the injection of a large i.v. dose of the DMS salt.

2.3. Anesthesia

Animals were fed until the evening prior to surgery and given tap water ad libitum until the time of the experiment. The animals were not premedicated. After weighing, all animals underwent an isoflurane (Abbott Laboratories Ltd., Montreal, Quebec, Canada) inhalation induction in the transport sling. Post-induction, the animals were placed in the dorsal recumbent position on an operating table and intubated with a 6.5 mm internal diameter (ID) cuffed oral endotracheal tube (Ruschelit, Willy Rusch, Kernen, Germany). Core body temperature was maintained at approximately 38.5 °C with a Therm-o-matic^{TM} heated operating table (Sage-London Industries Inc., London, Ontario, Canada). Rectal temperature was monitored continuously. Venous access was established by inserting a 22 gauge i.v. catheter into an ear-vein. A 20 gauge i.v. catheter was inserted into either femoral artery for blood pressure measurement and blood sampling.

All animals received normal saline i.v. (sodium chloride 0.9%, Baxter Corporation, Toronto, Ontario, Canada) at a rate of 9.2 ± 0.50 ml kg^{-1} h^{-1} (mean ± S.D.) via a volumetric infusion pump (Travenol FloGard 8000, Travenol Laboratories, Deerfield IL, U.S.A.). Capnography and pulse oximetry were performed (Nellcor N1000, Nellcor Inc., Hayward, CA). Data were collected with a Coulbourn Instruments LabLinc S computer interface (Coulbourn Instruments, Inc., Allentown, PA) to a Dell Optiplex 590 IBM compatible PC. Data were displayed and stored using WinGraph for windows software (Coulbourn Instruments). Respiratory variables were monitored using a Bicore CP 100 pulmonary monitor (Bear Medical Systems, Inc. Riverside, CA), which was, in turn, connected to a Dell Optiplex 590 IBM compatible PC. Data were displayed and stored using custom programmed software (Pulmonary Monitor 1.01, Black Cat Software, Calgary, Alberta, Canada). After all of the physiological measurment devices were secured, the animals were stabilized for at least 30 min to establish steady-state anesthesia (SSA). Continuous baseline physiological parameters were obtained and biochemical values were obtained prior to and post drug administration as described for the duration of anesthesia. Samples for clinical chemistry, hematology, and electrolytes were obtained prior to induction of anesthesia (i.m. HI-6 injections) or at SSA (i.v. HI-6 injections) and 5 h post HI-6. The samples were analyzed using an IDEXX VetTest Dry Chemistry Analyzer, VetLyte Electrolyte Analyzer and QBC AutoRead Hematology Analyzer (IDEXX Laboratories, Westbrook, Maine). Samples for blood gas analysis were drawn at SSA and 1, 3 and 5 h following HI-6 injection, and immediately analyzed on a Radiometer ABL5 Blood gas analyzer (London Scientific Limited, was prepared by centrifugation and frozen at −20 °C. The high i.v.dose of HI-6 (1899 mg i.v.) was administered in 3 ml saline rapidly into the ear vein.

2.4. Organophosphorous nerve agents and drug preparation

Soman or GF were synthesized in the single small scale facility at DRDC suffield. The GF (cyclohexyl methyl phosphonoflouridate) and GD (pinacolyl methylphosphonofluoridate) used in these studies were found to be more than 98% pure as determined by GC/MS and NMR. The agents were stored in vials at −50 °C (Sanyo ultra low freezer) and diluted in saline as required. Atropine and HI-6 were prepared in physiological saline just prior to the experiments and were injected in various doses to reach 1 ml/kg body weight i.m.

2.5. Preparation of HI-6 DMS

HI-6-dichloride (1-[[[4-(aminocarbonyl)-pyridio] methoxy]methyl]-2-[(hydroxyimino)methyl]pyridini-

um dichloride) was synthesized by Astra Pharmaceutical Chemicals (Sodertaje Sweden) and converted to the DMS salt by the method described by Thiermann et al. (1996b). A Dowex 1X2-200 ion exchange resin (100 g) was washed twice with a 1 M NaOH solution until no chloride ion could be detected (silver nitrate assay). The column was rinsed with water until the pH 8 was reached. The resin was converted to the methanesulphonate by eluting with 1 L of 1 M methyl sulfonic acid followed by water until the effluent pH was neutral.

HI-6 dichloride (25 g) dissolved in 150 ml of water was applied to the column and eluted with a 0.5 mM methyl sulfonic acid solution. The first 50 ml fraction contained chloride ions. The next six fractions of 50 ml were combined and lyophilized. The yellow solid was recrystallized from water/ethanol, collected and dried under vacuum. 19.57 g of HI-6 dimethylsulfonate were isolated. Purity of the DMS salt was determined by elemental analysis and found to be free of chloride ions. All chemicals used in this process were purchased from Aldrich. Water was double distilled before use.

2.6. LD_{50} determination in guinea pigs

LD_{50} determinations have been historically carried out in this laboratory using standard probit tests. Confirmation of previous LD_{50} values (Lundy et al., 1989) was carried out by the up and down method of Dixon (1965).This method has the added benefit of reducing animal use and the LD_{50}'s were calculated after 6 h to adhere to the spirit of animal rights legislation and also because results obtained at 6 h or the more traditional 24 h, in our experience, have been very similar. Soman or GF were injected s.c. in the neck. The LD_{50} values obtained were very similar to those reported previously from this and other laboratories.

2.7. Injection of HI-6 or HI-6 plus atropine in swine

Either salt of HI-6 (500 mg HI-6 2Cl or 633 mg HI-6 DMS) was prepared in saline to deliver a volume of 2 ml and injected into the gluteus muscle. Control blood samples were drawn into EDTA tubes from the arterial catheter and additional samples were drawn at various time intervals up to 6 h following HI-6 or HI-6/atropine injections. Plasma was prepared by centrifugation and frozen at − 20 C. The high i.v.dose of HI-6 (1899 mg i.v.) was administered in 3 ml saline rapidly into the ear vein.

2.8. HPLC determination of HI-6 in plasma

HI-6 was measured based on previous published methods (Hamilton and Lundy, 1989), with the following modifications. Samples were analyzed on an HP Chemstation 1100 HPLC following a 5 ul sample injection. The mobile phase was 7% acetonitrile in 0.1 M phosphate buffer, pH 2.5 (3.42 ml/L concentrated H_3PO_4, 13.6 g/L KH_2PO_4), 75 mg/L EDTA, 60 mg/L 1-octanesulfonic acid. The stationary phase used was Zorbax Reliance ODS column (6.0 mm × 40 mm, 3 μ). Samples were run at 1 ml/min and 30 C. Absorbance readings were taken at 304 nm. HI-6 concentrations were determined by comparison of the ratio of HI-6 peak area/internal standard peak area to a standard curve.

2.9. Efficacy of HI-6 DMS in guinea pigs for the treatment of soman and GF

Immediately following the injection of the nerve agent (s.c.) atropine (17 mg/kg i.m.) alone or atropine plus various doses of HI-6 DMS (1 mg/kg–172 mg/kg i.m.) were injected i.m. into either hip.The animals were periodically examined over the 6 h experimental period for signs of poisoning and lethality

2.10. Experimental design

The pharmacological activity of both doses of HI-6 DMS (633 mg/kg i.m. and 1899 mg i.v.) and the 500 mg i.m. dose of HI-6 2Cl were measured and compared with one another to examine the possibility that (a) the DMS salt might produce effects different from those seen with the 2Cl salt at equimolar concentrations and (b) to examine the continuously recorded pharmacodynamic effects of both the smaller doses given under similar conditions and the very high i.v. dose of DMS. Blood samples were drawn at 1, 3, 5, 10, 15, 30, 60 min and every 30 min until 4 h or 6 h following the i.v. or i.m. dose respectively while at the same time pharmacodynamic responses were recorded so that they could be co-related with plasma HI-6 concentrations obtained from the pharmacokinetic studies. Pharma-

codynamic responses measured included systolic diastolic and mean arterial blood pressure, heart rate, respiratory rate, minute ventilation, end tidal CO_2, arterial oxygen saturation and body temperature.

Guinea pigs were treated with ONA and atropine plus HI-6 as has been carried out previously in this laboratory (Lundy et al., 1989, 1992) so that some general comparisons could be made with the animals treated at this time with HI-6 DMS and those treated previously with atropine HI-6 2Cl

2.11. Statistical analysis

The magnitude of the differences in the physiological responses were determined by (ANOVA) and then Bonferroni test applied. Significance as in the case of the change in arterial blood pressure was considered significant $p < 0.05$

3. Results

3.1. Pharmacokinetics of HI-6 in swine

Fig. 1 A shows the plasma HI-6 concentrations measured following the injection of 500 mg of HI-6 2Cl i.m. compared with values obtained following injection of the molar equivalent of HI-6 DMS (633 mg i.m.) in domestic swine. The points on Fig. 1B represents plasma concentrations of HI-6 at various time periods following injection of HI-6 in the presence of 2 mg atropine. The two curves representing the plasma concentrations of HI-6 following the injection of either salt were not statistically different from each other at any of the time periods examined. Co-administration of atropine failed to significantly alter plasma HI-6 concentrations. Moreover, the pharmacokinetic constants that were calculated (Table 1) are identical and appear to be remarkably similar to values obtained by other investigators following administration of HI-6 2Cl in pigs (Göransson-Nyberg et al., 1995).

A group of animals were given an i.v. dose of HI-6 DMS (1899 mg) which was three times the dose given by intramuscular injection. Plasma HI-6 concentrations measured following the i.m. administration of the smaller dose and the i.v. administration of the high dose are found on Fig. 2. In this figure the average peak plasma level following the i.m. injections was

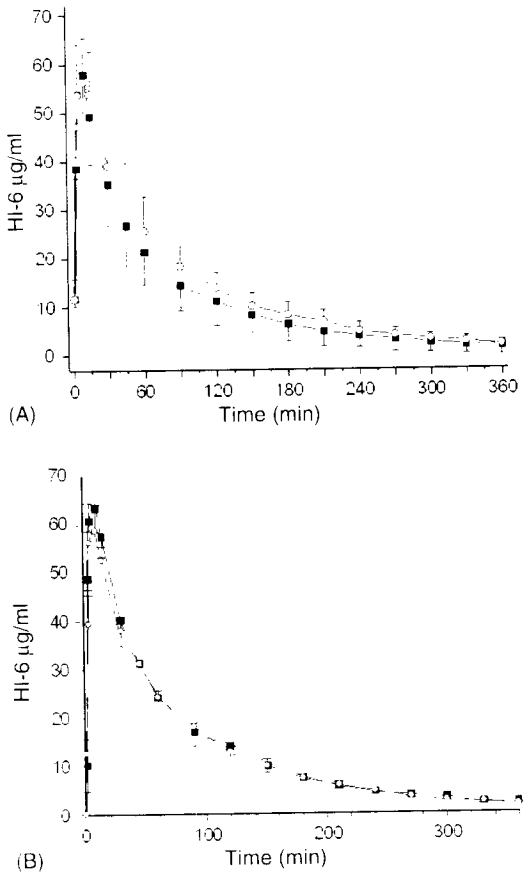

Fig. 1. (A) Representation of the absorption and distribution characteristics of HI-6 following the i.m. administration of equimolar doses of the dichloride (500 mg, ■) or the DMS salt in juvenile swine (633 mg, □). (B) Represents the pharmacokinetic parameters of HI-6 in pigs given HI-6 DMS, 633 mg, (□) in the presence of atropine sulphate 2 mg i.m. (■). Anesthetised swine were injected and blood samples collected at various time intervals from 1 min to 6 h and HI-6 concentrations determined by HPLC. Each point on the graph represents the mean HI-6 concentration ± S.E.M. ($N = 6$).

63 µg/mL and the average plasma level following the i.v. injections was 761 µg/mL.

3.2. Pharmacodynamics of HI-6 in swine

As evidenced in Figs. 3–5 there were no clinically significant alterations in minute ventilation (3A), end tidal CO_2 (3B), heart rate (4A), mean arterial pressure (4B), arterial O_2 saturation (5A) or body temperature (5B). The lack of effect of 500 mg of HI-6 2Cl in small swine of this type has been previously demonstrated

Table 1
Pharmacokinetic data obtained following a single i.m. injection of HI-6 2Cl 500 mg i.m.or HI-6 DMS 633 mg. i.m. in swine

Parameter	HI-6-2Cl	HI-6-DMS
Half-life (min)		
Absorption phase	2.00 ± 0.14	2.20 ± 0.66
Distribution phase	12.59 ± 2.44	20.34 ± 6.08
Elimination phase	81.45 ± 17.23	82.87 ± 5.35
C_{max} (μg/mL)	60.13 ± 3.34	62.82 ± 3.68
T_{max} (min)	9.17 ± 1.54	9.17 ± 1.54
AUC_{inf} (μg/mL min)	4457 ± 632	5131 ± 450
V_d (L/kg)	0.53 ± 0.07	0.49 ± 0.05
CL (ml/min/kg)	5.10 ± 0.90	4.13 ± 0.47

All values are mean ± S.E.M. ($N = 6$). The values for each salt do not statistically differ with respect to any parameter (student's t-test). C_{max} = max plasma conc.HI-6; AUC = area under curve; CL = clearance; T_{max} = time max plasma conc; V_d = Volume of distribution.

(Göransson-Nyberg et al., 1995). Fig. 4B shows, however, that the intravenous dose of 1899 mg DMS (when the plasma HI-6 concentration reached 761 μg/mL) resulted in about a 20% reduction in arterial pressure of short duration. No significant effects on biochemistry, hematology, electrolytes or blood gasses were observed following HI-6 dichloride or DMS at any dose in the presence or absence of atropine (results not shown).

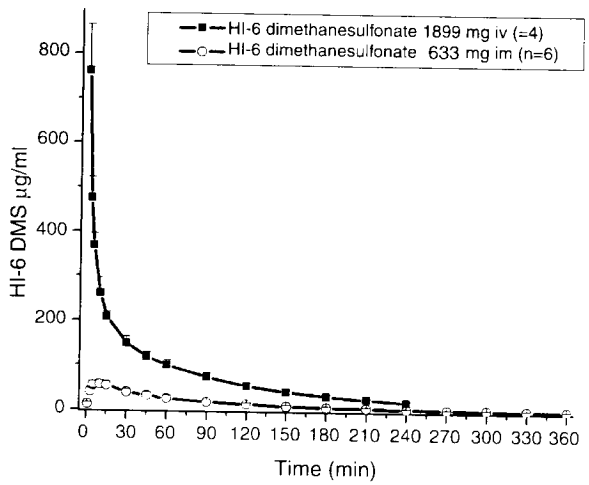

Fig. 2. The comparison of plasma HI-6 concentrations for various time intervals from 1 min to 6 h following either an i.m. injection of 633 mg (◯) HI-6 DMS or an i.v. dose 1899 mg (■) in anesthetized swine. Each point represents the mean plasma concentration of HI-6 ± S.E.M. from either four (i.v. route) or six (i.m. route) animals.

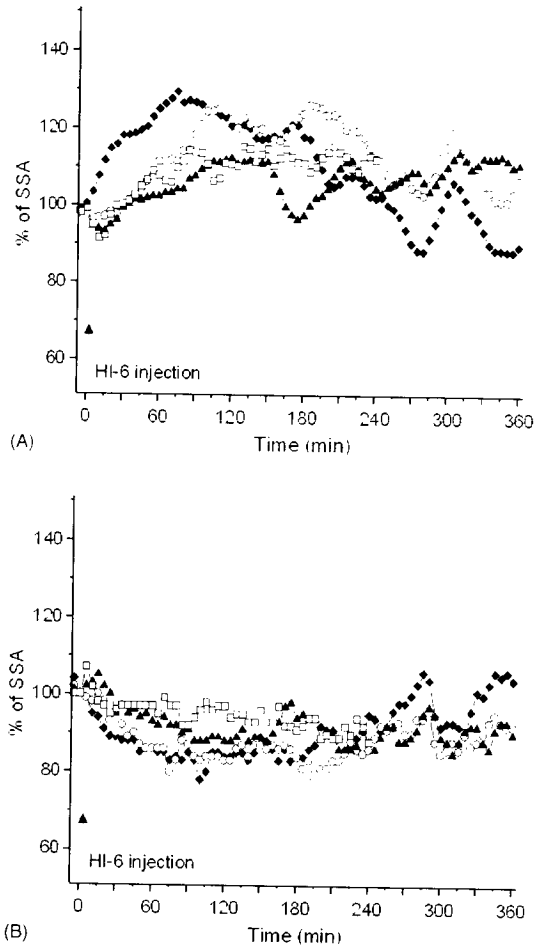

Fig. 3. A summary of the effects of either 500 mg HI6 2CL i.m. (◯), HI-6 DMS 633 mg (▲) or 1899 mg HI-6 DMS i.v. (□) as compared to controls (■) (steady state anesthesia -SSA) on minute volume (A) or end tidal CO_2 (B). Each point is the mean of results obtained in six animals (i.m doses) or four animals (i.v dose).

3.3. *Efficacy of HI-6 DMS against GF and soman poisoning*

In these experiments all animals were given atropine (17 mg/kg) (Lundy et al., 1989) in conjunction with HI-6. HI-6 2Cl at the dose selected here has been previously shown to be extremely effective against GF poisoning in guinea pigs and, relative to other oximes, to be very effective against soman poisoning as well (Lundy et al., 1992). The effectiveness of HI-6 DMS appears not unexpectedly to be demonstrated in a dose dependant fashion which are results similar to results

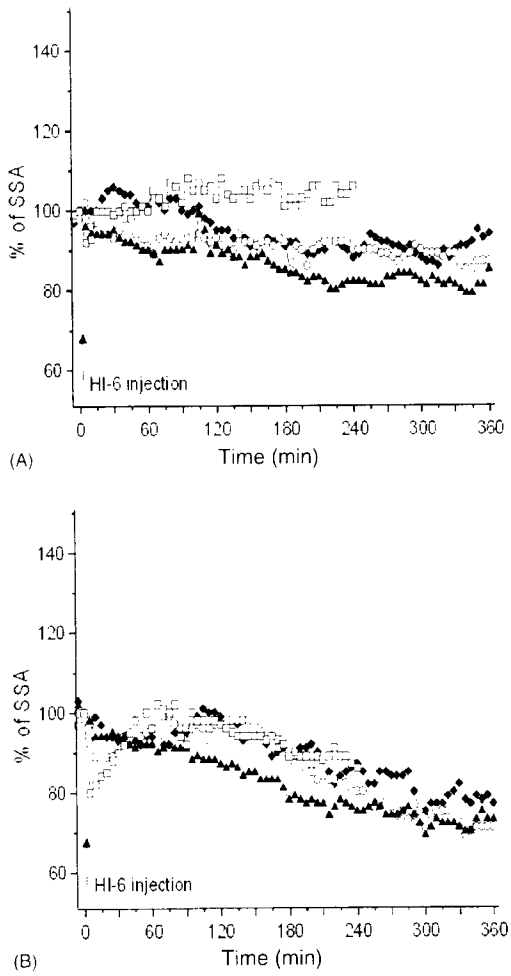

Fig. 4. (A) Changes in heart rate and blood pressure (B) at various time intervals up to 6 h following the injection of 500 mg HI6 2CL i.m (○) compared with effects of HI-6 DMS 633 mg i.m. (▲) or i.v. HI-6 DMS 1899 mg (□) as compared to controls (steady state anesthesia-SSA). Each point represents the mean value of six animals in the i.m. groups or four animals in the i.v. group.

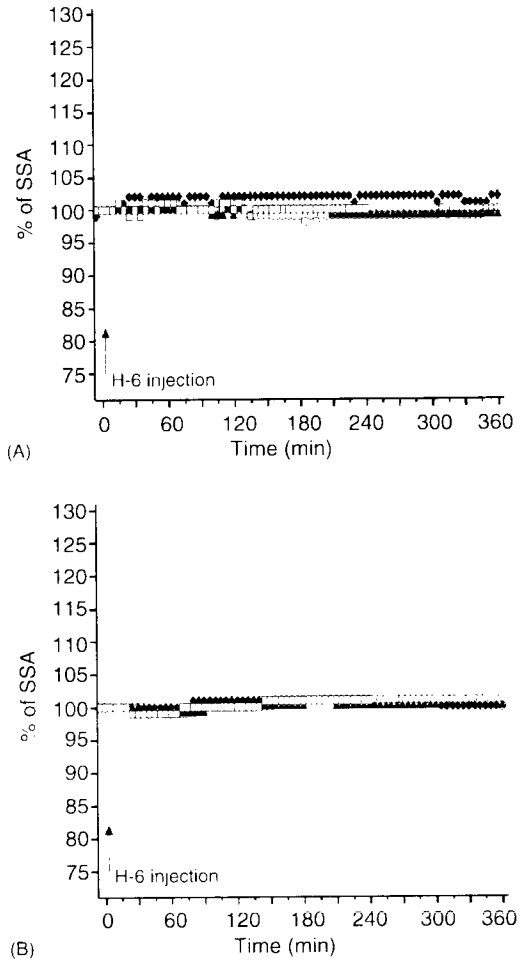

Fig. 5. The changes in arterial oxygen saturation (A) and body temperature (B) following three different HI-6 treatments. Changes induced by either HI-6 2Cl 500 mg i.m. (○); HI-6 DMS 633 mg i.m. (▲) or HI-6 DMS 1899 mg i.v. (□) are compared to controls (■ during steady state anesthesia-SSA). Each point represents the mean value obtained from six animals following the i.m dose or four animals following the i.v dose.

obtained with the 2Cl salt (Maxwell and Koplovitz, 1990). All guinea pigs exposed to $5 \times LD_{50}$ of either soman or GF were protected by HI-6 DMS. The protection afforded was both dose and agent dependent. Atropine sulfate alone failed to protect any animal from $2 \times LD_{50}$ of either agent. It is clear from results shown in Fig. 6 that a dose of 3 mg/kg HI-6 DMS was sufficient to protect 50% of the animals from $5 \times LD_{50}$ of GF whereas a dose of 154 mg/kg was required to effect this protection in soman poisoned animals. The maximal protective effects of HI-6 DMS were not examined in the present studies but in unpublished experiments we have been able to protect animals from at least $30 \times LD_{50}$ of GF.

4. Discussion

As pointed out in the introduction, HI-6 2Cl 500 mg, the amount of drug currently supplied in the available

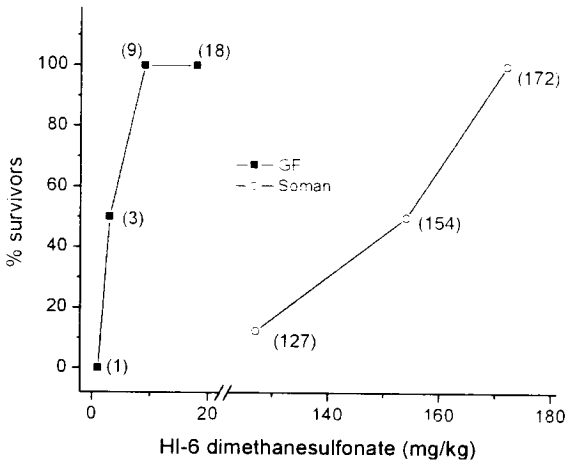

Fig. 6. The percentage survival of guinea pigs given 5 × LD_{50} s.c. of either GF (■) or soman (□). Administration of organophosphate cholinesterase inhibitors was followed immediately by atropine sulfate (17 mg/kg i.m.) and various doses of HI-6 DMS (1–18 mg/kg i.m. against GF or 127–176 mg/kg i.m. against soman). The survival rate was determined 6 h following poisoning.

autoinjectors, was not chosen as the result of human toxicity concerns (Kušic et al., 1985, 1991; Clement et al., 1995; Jovanovic et al., 1990), but rather because 500 mg approached the limit of solubility in the restricted volume of these devises (Thiermann et al., 1996b). In fact, in the human trials carried out to date a toxic dose of HI-6 in man has not yet been identified. As has been clearly pointed out, the DMS salt is much more soluble (up to 6 times depending on temperature) than the 2Cl salt, particularly at lower environmental temperatures (Thiermann et al., 1996b), a finding which would be consistent with the inclusion of much larger dose of HI-6 in the injector, if it could be determined that larger doses of HI-6 would be more efficacious.

Although HI-6 2Cl has been safely used in humans (Kušic et al., 1985; Jovanovic et al., 1990; Kušic et al., 1991; Clement et al., 1995), HI-6 DMS has yet to be used in man. The studies presented here were carried out for three major reasons based on the desire to eventually obtain regulatory approval for the DMS salt of HI-6 by comparing several aspects of its pharmacology/safety and efficacy to the more extensively studied 2Cl salt. The first objective was to compare the pharmacokinetics and the pharmacodynamics of the two salts of HI-6. The second objective was to determine whether the DMS salt demonstrated protective effects consistent with those published in the literature for the 2Cl salt and the third was to examine the dose related protective effects of HI-6 against oxime resistant nerve agents in order to determine whether larger doses of HI-6 could be recommended for examination in future clinical trials and ultimately inclusion in the autoinjector. The demonstration of definitively increased protective effects of increasing doses of HI-6 against these two oxime resistant ONA's would suggest clearly that higher doses of HI-6, than 500 mg which could be attained through the use of the DMS salt, should be considered for inclusion in the autoinjector.The effectiveness of these high doses suggests that perhaps separate formulations should be developed for use for i.m. or i.v. injection under medical direction as required in clinical settings as the result of terrorist activity for example.

4.1. Pharmacokinetic/Pharmacodynamic studies

The studies revealed that at equimolar doses (500 mg 2CL or 633 mg DMS) the two salts of HI-6 displayed an identical pharmacokinetic profile (Table 1). The co-administration of atropine had no effect on the pharmacokinetics of HI-6, findings similar to results obtained with atropine HI-6 reported in other species (Spohrer et al., 1994; Clement et al., 1995). The pharmacokinetics of HI-6 2Cl have been extensively studied in a variety of species, including pig, dog and man. The results obtained in the present study following the injection of a 500 mg dose of the 2Cl salt are consistent with previous studies in pigs (Goransson-Nyberg et al., 1995) and dogs (Klimmek and Eyer, 1986; Thiermann et al., 1996a), and therefore, the derived pharmacokinetic parameters suggest considerable cross species similarity. The similarity between the DMS and the 2Cl salt in pigs as well as the similarity between the pharmacokinetics of HI-6 2Cl among different species, strongly suggests that the DMS and 2 Cl would be expected to have a similar profile to HI-6 2Cl in man as well.

The high dose of HI-6 DMS (1899 mg/kg i.v.) resulted in a statistically significant decrease in arterial pressure lasting for 20 min followed by a return to anesthetic control values where it remained for the duration of the experiment. This decrease co related generally with the times at which the plasma HI-6 concentrations were at their highest. Very high i.v.doses of HI-

6 DMS (or of HI-6 2Cl) have previously never been examined with respect to any of the physiological parameters measured here. In primates, however Amitai et al. (1995) have reported the safe use of very high doses of HI-6 2Cl (225–346 mg/kg i.m.) and observed only minor adverse side effects, however changes in cardiovascular effects were not measured in these studies (Amitai et al. 1995; Amitai personnal communication). Humans treated with up to 500 mg of the 2Cl salt which produced plasma concentrations far below those reported here (15–20 μg/ml versus > 700 μg/ml), suffered no alteration in any cardiovascular parameters measured (Kusic et al.1995, Clement et al., 1994). Transient small decreases in arterial pressure have been reported in guinea pigs (De La Motte and Szinicz, 1991). It is also clear that in organophosphate poisoned guinea pigs, HI-6 failed to add to OPA induced hypotension but rather reversed it especially in combination with atropine (see Worek and Szinicz, 1993b). These effects on arterial blood pressure are presumably the result of the weak ganglionic blocking properties of HI-6 (Lundy and Tremblay, 1979).

Significant alterations in clinical chemistry parameters were also absent in pigs given DMS or primates given 2Cl in high doses (Lundy et. al. unpublished observations, Amitai, personnal communication) suggesting little adverse effects of HI-6 on liver and other vital organ function. These observations are important with respect to the determination of an effective human dose of HI-6. It is also clear from the literature that doses of HI-6 which were demonstrated to be protective against large nerve agent doses ($5 \times LD_{50}$ soman) in primates, were in the range of 50 mg/kg (Hamilton and Lundy, 1989; van Helden et al., 1992; Amitai et al., 1995; Busker et al., 1996), or even much higher in some cases (Amitai et al., 1995; Chen et al., 2001). These doses and the plasma concentrations attained are all considerably larger than doses currently suggested for human use. Primate doses of HI-6 of about 50–60 mg/kg have been reported to produce protection against supra lethal doses of soman and to produce plasma levels of oxime as high as 170–180 μg/ml (van Helden et al., 1992; Busker et al., 1996). Much higher plasma levels of HI-6 were obtained following high dose administration carried out in baboons (Amitai et al., personnal communication). The dose of HI-6 currently proposed for human use from one autoinjector (which contains HI-6, 500 mg), produced peak plasma levels of only about 15 μg/mL in man (Clement et al., 1995; Kušic et al., 1985). Even if three autoinjectors were used in man (1500 mg or 22 mg/kg) one could project a peak plasma concentrations of about 50 μg/mL. Therefore, the present human dose delivered by three autoinjectors could be logically expected to produce plasma concentrations much less than those resulting in protection in the primate and guinea pig studies referred to earlier, particularly when the agent of concern was soman (see discussion of next section). It is also clear that very high blood concentrations of HI-6 failed to result in significant toxic signs in pigs and primates.

4.2. *Efficacy studies*

As pointed out in the introduction, the third objective for carrying out this study was to examine the efficacy of HI-6 DMS in a relevant species. These studies were carried out in guinea pigs, a standard model which reflects accurately the responses to nerve agents and nerve agent therapies in primates and therefore presumably in man. Two agents, GF and soman, which are notoriously resistant to presently licensed oximes (Lundy et al., 1992) were chosen to examine the protective effects of HI-6 DMS. There was a clear and dramatic dose related protection by HI-6 DMS against soman and GF. HI-6 DMS dose dependantly increased protection against $5 \times LD_{50}$ of soman. A similar pattern of dose related protection was observed against a $5 \times LD_{50}$ of GF. There was however a marked difference in the dose of HI-6 DMS which was effective against the two nerve agents.

The dose dependant protection produced by HI-6 DMS in the present study was similar to dose dependant protection previously demonstrated for HI-6 2Cl against tabun, soman and GF (Maxwell and Koplovitz, 1990; Worek and Szinicz, 1993b; Maxwell et al., 1997; Lundy et al., 1992). It is also clear that there are extreme differences in the dose of oximes required to treat different nerve agents. The dose required to protect animals from $5 \times LD_{50}$ of GF appears to be about 50-fold lower than required to protect animals from equally toxic doses of soman. The maximally effective doses reported here and reported by Amitai et al. (1995) are higher than doses routinely used by other investigators who have examined the protective effects of HI-6 dichloride (for example see Worek and Szinicz, 1993a;

Maxwell et al., 1997). HI-6 has also been demonstrated to produce dose dependant protection against lethality and improved the clinical status of baboons treated with high doses of tabun (Amitai et al., 1995). The usefulness of these high doses must be considered in the selection of a dose which might be recommended either for inclusion in the autoinjectors or for i.m. or i.v. administration of HI-6 under medical supervision to man, rather only as first aid (see below). These studies appear to suggest that higher doses of HI-6 than those presently available for human use (22 mg/kg) might be well tolerated as well as be more effective in treating human nerve agent casualties. The validity of suggestions await further clinical trials.

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References

Amitai, G., Rabinovitz, I., Zomber, G., Chen, R., Cohen, G., Adani, R., Raveh, L., 1995. Bisquaternary oximes as antidotes against tabun and soman poisoning. In: Quin, D.M., et al. (Eds.), Antidotal efficacy in relation to cholinesterase reactivation. Enzymes of the Cholinesterase family. Plenum Press, N.Y.

Busker, R.W., Zijlstra, J.J., Philippens, I.H.C.H.M., Groen, B., Melchers, B.P.C., 1996. Comparison of the efficacy of single or repeated HI-6 treatment following soman poisoning in guinea pigs and marmoset monkeys. Toxicology 112, 183–194.

Chen, R., Raveh, L., Zomber, G., Rabinovitz, I., Cohen, R., Adani, R., Amitai, G., 2001. Determination of therapeutic doses of bisquaternary oximes in large animals. J. Appl. Toxicol. 21, 285–291.

Clement, J.G., 1992. Efficacy of various oximes against GF (cyclohexyl methylphosphonoflouridate) poisoning in mice. Arch. Toxicol. 66, 143–144.

Clement, J.C., Bailey, D.G., Madill, H.D., Tran, L.T., Spence, J.D., 1995. The acetylcholinesterase reactivator HI-6 in man: Pharmacokinetics and tolerability in combination with atropine. Biopharm. Drug Dist. 16, 415–425.

De La Motte, S., Sziniez, L., 1991. Effects of pyridinium, 1{[4-carbamoyl-pyridino) methoxy]methyl}-2-(hydroxyiminomethyl) dichloride monohydrate (HI6) and atropine on the circulation and respiration of anesthetized guinea pigs. In: Recent Developments in Toxicology: Trends Methods and Problems. Arch. Toxicol., Suppl. 14, pp. 266–268. Springer Verlag.

Dixon, W.J., 1965. The up-and-down method for small samples. Am. Stat. Assoc. J., 967–978.

Göransson-Nyberg, A., Cassel, G., Jeneskog, T., Karlsson, L., Larsson, R., Lundstrom, M., Palmer, L., Persson, S.-A., 1995. Pharmacokinetics of HI-6 and atropine in anesthetized pigs after administration by a new autoinjector. Biopharm. Drug Dist. 16, 915.1–915.17.

Göransson-Nyberg, A., Cassel, G., Jeneskog, T., Karlsson, L., Larsson, R., Lundström, M., Persson, S.-A., 1995. Treatment of organophosphate poisoning in pigs: antidote administration by a new binary autoinjector. Arch. Toxicol. 70, 20–27.

Hamilton, M.G., Lundy, P.M., 1989. HI-6 therapy of soman and tabun poisoning in primates and rodents. Arch. Toxicol. 63, 144–149.

Jovanovic, D., Randelovic, S., Joksovic, D., 1990a. A case of unusual suicidal poisoning by the organophosphorous insecticide dimethoate. Hum. Exp. Toxicol. 9, 49–51.

Jovanovic, D., Maksimovic, M., Joksovic, V., 1990b. Oral forms of the oxime HI-6: a study of pharmacokinetics and tolerance after administration to healthy volunteers. Vet. Hum. Toxicol. 32, 419–421.

Kassa, J., Bajgar, J., 1995. Comparison of the efficacy of HI-6 and obidoxime against cyclohexylmethylphosphono-flouridate (GF) in rats. Hum. Exp. Toxicol. 14, 923–928.

Klimmek, R., Eyer, P., 1986. Pharmacokinetics and pharmacodynamics of the oxime HI-6 in dogs. Arch. Toxicol. 59, 272–278.

Krummer, S., Thiermann, H., Worek, F., Eyer, P., 2002. Equipotent cholinesterase reactivation in vitro by the nerve agent antidotes HI-6 dichloride and HI-6 dimethanesulfonate. Arch. Toxicol. 76, 589–595.

Kušic, R., Bosković, B., Vojvodić, V., Jovanović, D., 1985. HI-6 in man: blood levels, urinary excretion, and tolerance after intramuscular administratuion of the oxime to healthy volunteers. Fund. Appl. Toxicol. 5, s89–s97.

Kušic, R., Jovanović, D., Randjelovic, S., Joksovic, D., Todorovic, V., Boskovic, B., Jokanovic, M., Vojvodic, V., 1991. HI-6 in man: efficacy of the oxime in poisoning by organophosphate insecticides. Hum. Exp. Toxicol. 10, 113–118.

Lundy, P.M., Tremblay, K., 1979. The ganglionic blocking properties of some bispyridinium soman antagonists. Eur. J. Pharmacol. 60, 47–53.

Lundy, P.M., Goulet, J.-C., Hand, B.T., 1989. Hormone and dose schedule dependant protection by HI-6 against soman and tabun poisoning. Fund. Appl. Toxicol. 12, 595–603.

Lundy, P.M., Hansen, A.S., Hand, B.T., Boulet, C.A., 1992. Comparison of several oximes against poisoning by soman tabun and GF. Toxicology 72, 99–105.

Maxwell, D.M., Koplovitz, I., 1990. Effect of endogenous carboxylesterase on HI-6 protection against soman toxicity. J. Pharmacol. Exp. Ther. 254, 440–444.

Maxwell, D.M., Brecht, K.M., Koplovitz, I., 1997. Characterization and treatment of the toxicity of *O*-isobutyl S-[2-(diethylamino)ethyl]methylphosphonothioate, a structural analogue of VX in guinea pigs. J. Am. Col. Toxicol. 15, S78–S88.

Spohrer, U., Thiermann, H., Klimmek, R., Eyer, P., 1994. Pharmacokinetics of the oximes HI-6 and Hlo7 in dogs after i.m. injection

with newly developed dry/wet autoinjectors. Arch. Toxicol. 68, 480–489.

Thiermann, H., Radtke, M., Spohrer, U., Klimmek, R., Eyre, P., 1996a. Pharmacokinetics of atropine in dogs after i.m. injection with newly developed dry/wet combination autoinjectors containing HI 6 or Hlo 7. Arch. Toxicol. 70, 293–299.

Thiermann, H., Seidl, S., Eyer, P., 1996b. HI-6 dimethanesulfonate has better dissolution properties than HI 6 dichloride for application in dry/wet autoinjectors. Int. J. Pharm. 137, 167–176.

van Helden, H.P.M., Van der Wiel, H.J., De Lange, J., Busker, R., Melchers, B.P.C., Wolthuis, O.L., 1992. Therapeutic efficacy of HI-6 in soman-poisoned marmoset monkeys. Toxicol. Appl. Pharmacol. 115, 50–56.

Worek, F., Szinicz, L., 1993a. Atropine and oxime treatment in lethal soman poisoning of anesthetized guinea pigs: Hlo7 dimethanesulfonate versus HI-6 dichloride. Pharmacol. Toxicol. 72, 13–21.

Worek, F., Szinicz, L., 1993b. Investigation of acute cardiovascular and respiratory toxicity of Hlo7 dimethanesulphonate and HI 6 dichloride in anesthetized guinea-pigs. Pharmacol. Toxicol. 73, 91–95.

Worek, F., Widmann, R., Knopff, O., Szinicz, L., 1998. Reactivating potency of obidoxime, pralidoxime, HI-6 and HLö 7 in human erythrocyte acetylcholinesterase inhibited by highly toxic organophosphorous compounds. Arch. Toxicol. 72, 237–243.