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PHARMACOKINETICS AND PHARMACODYNAMICS OF OXIMES IN UNANESTHETIZED PIGS

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The pharmacokinetics and cardiovascular pharmacodynamics of three oximes were studied in unanesthetized pigs. Effects of 2-[(hydroxyimino)methyl]-1-methylpyridinium chloride (pralidoxime chloride; 2-PAM Cl; ICD 001; 50 umol/kg) were compared with those of 1,1-methylene bis[4(hydroxyiminomethyl) pyridinium] dichloride (methoxime; MMB-4; ICD 039; 100 umol/kg) and 2-hydroxyiminomethyl-3-methyl-1-(2-3-methyl-3-nitrobutyloxymethyl))-imidazolium chloride (ICD 467; 12.5 umol/kg). Cardiopulmonary parameters were monitored and plasma concentrations of oximes were determined from arterial blood samples taken at intervals over a period of 5 hr postinjection. Plasma concentrations for all oximes were fitted to standard pharmacokinetic models using the computer program PCNONLIN. Average pharmacokinetic parameters were determined for each oxime. Only mild to moderate physiological side effects were detected following intramuscular administration. 2-PAM Cl and ICD 467 were both more rapidly absorbed, with slightly faster distribution and elimination rates for ICD 467. Although MMB-4 had a slight lag time to attain detectable levels in the blood, retention time was longer than for 2-PAM Cl and ICD 467.

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PREFACE

The work reported herein was conducted under USAMRICD Protocol #1-03-89-000-A-518, entitled "Pharmacokinetics and Pharmacodynamics of Oximes Intramuscularly Administered to Unanesthetized Pigs." The data is recorded in USAMRICD notebook number 002-89. The work was initiated in January 1989 and completed in May 1989.

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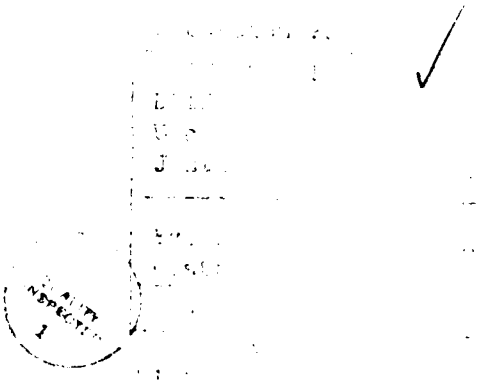

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INTRODUCTION

Current treatment for organophosphorus poisoning includes oxime therapy, pralidoxime chloride (2[(hydroxyimino) methyl]-1-methylpyridinium chloride; 2-PAM Cl; ICD 001; 50 μmol/kg) to reactivate inhibited acetylcholinesterase (AChE), and atropine therapy to antagonize muscarinic effects of excess acetylcholine (Fleisher *et al.*, 1970). We have identified two other oximes as potential improvements over 2-PAM Cl. These oximes, 1,1-methylene bis[4(hydroxyiminomethyl) pyridinium] dichloride (methoxime; MMB-4; ICD 039) and 2-hydroxyiminomethyl-3-methyl-1-(2-(3-methyl-3-nitrobutyloxy-methyl))-imidazolium chloride (ICD 467), possess greater efficacy than 2-PAM Cl against soman in mice (Koplovitz and Stewart, 1988). Additional testing was needed to assess efficacy and safety in non-rodent populations. Both MMB-4 and 2-PAM Cl are soluble in water, and the latter has low solubility in acetone, ethanol, and isopropanol indicating poor lipophilic characteristics (Banakar and Patel, 1988). Because of a chemical structure similar to that of 2-PAM Cl, MMB-4 would also possess poor lipophilic properties.

The rate of oxime absorption into the blood is a critical aspect of therapeutic efficacy. To evaluate an oxime as a candidate AChE reactivator, the aging phenomenon of inhibited AChE must be considered. Aging is defined as the progressive loss of the ability of phosphonylated AChE to be reactivated by oximes (Fleisher and Harris, 1965). Once aging has occurred, reactivation of inhibited AChE by oximes is not possible (Talbot *et al.*, 1988). The rate for aging of inhibited AChE varies with species and agents used (Harris *et al.*, 1966; Fleisher *et al.*, 1967; Talbot *et al.*, 1988).

Talbot *et al.* (1988) determined that in primate erythrocytes the *in vivo* half-life of AChE aging following soman exposure was approximately 1 min and approximately 8 min in rat and guinea pig erythrocytes, respectively. *In vitro* studies in the erythrocytes from marmosets and guinea pigs showed enzyme aging times that were almost identical to *in vivo* times (Talbot *et al.*, 1988). If these *in vitro*/*in vivo* responses to soman are applicable to humans, the rate of human erythrocyte AChE aging *in vitro* should provide a good estimate of *in vivo* aging. *In vitro* human erythrocyte AChE aging was determined by Harris *et al.* (1978) to be 1.3 min.

In view of the rapid aging for soman-inhibited AChE in man, oximes (especially 2-PAM Cl) may not be an important adjunct in the treatment of poisoning, because 75% of the inhibited enzyme would probably be aged (2 min) before symptoms appear and oxime therapy is administered. Furthermore, the concentration achieved in the blood by 2-PAM Cl administration is inadequate for reactivation of unaged soman-inhibited AChE (Fleisher *et al.*, 1967). Thus, a replacement oxime for 2-PAM Cl is needed for the Armed Forces.

The objectives of this study were to investigate the pharmacokinetics of the three compounds and to determine their acute cardiopulmonary effects in pigs. Since the pig model was new to this laboratory and little information was available with respect to the effects of oximes in the pig, 2-PAM Cl was included in this study as a reference chemical for MMB-4 and ICD 467. The dose of 2-PAM Cl in this study was selected to be comparable with clinical doses. The dose of MMB-4 (100 μmol/kg) selected for pigs was approximately equivalent to the maximum dose deliverable to a 70 kg human dose in 6 ml (three 2-ml autoinjectors). For the purpose of this study, the maximum test dose

was twice the maximum man-equivalent dose. In a pilot study with pigs injected with maximum human-equivalent doses of MMB-4, no overt symptoms of toxicity were observed during a 2-hr period of observation. The dose for ICD 467, also determined in a pilot study, was 12.5 μmol/kg.

METHODS

Animals. Sixteen castrated Chester White-Yorkshire Cross male pigs (*Sus scrofa*) weighing between 16 and 22 kg were used in these experiments (n=8 per group). Pigs from a commercial breeder were maintained in quarantine for 7 days in indoor/outdoor concrete runs while they were screened for evidence of disease. They were then individually housed inside in 4 x 6-foot pens with stainless steel sides and slatted aluminum floors. The room was maintained at 20-22°C, relative humidity of 50 ± 4.10%, on a 12-hr light/dark cycle with no twilight. Tap water was provided *ad libitum*, and Lab Porcine Chow Grower (Purina 5084, Purina Mills, Inc., Richmond, IN) was hand fed twice a day. Bananas were mixed with the food as an enticement. The pigs were touched and stroked while feeding to minimize stress during subsequent handling. Daily training sessions (initially 0.5 hr and gradually increased to a maximum of 2 hr) were conducted to familiarize pigs with attendants and to condition the pigs to restraint in a Panepinto sling (Panepinto, 1986). The limbs were loosely tied when the unanesthetized pigs were positioned in the sling. The pigs were trained to accept a cone which covered the nose and mouth and was attached to a pneumotachograph for measuring respiratory air flow. Training sessions continued for 7-10 days pre- and 1-2 days postsurgery. Pigs were used because their size allowed for the collection of large amounts of blood for analyses and for making cardiopulmonary measurements. Pigs are regarded as an appropriate physiologic model for humans because of similarities in many swine and human functions, e.g., cardiovascular and pulmonary (Phillips and Tumbleson, 1986).

Surgery. After an overnight fast, each pig received intramuscular (im) injections of 0.08 mg/kg atropine sulfate, 2.2 mg/kg ketamine hydrochloride, and 2.2 mg/kg xylazine. An endotracheal tube (5.0 mm o.d.) was inserted, and anesthesia was maintained with a mixture of halothane, oxygen, and nitrous oxide. A polyvinyl catheter (0.28 cm o.d. / 0.17 cm i.d.) was inserted into the left carotid artery through a ventral paramidline cervical incision and advanced into the aorta. A Gould heparin-coated flow-directed thermodilution catheter (5F) was inserted into the left internal jugular vein and advanced through the right atrium and ventricle into the pulmonary artery. Both catheters were tunneled subcutaneously to the dorsal midline, exited the skin through a common opening, and were filled with sodium heparin (1000 U/ml). The ends of the catheters were placed between two Velcro patches, one of which was sutured to the skin. The catheters were cleared daily by withdrawal of fluid and refilled with fresh heparin. Ampicillin sodium (22 mg/kg iv) was administered daily to prevent postoperative sepsis.

Experimental procedures. Two days after surgery, the pig was placed in the Panepinto sling and a nose cone was mounted securely over the snout. The pig was then allowed a 45- to 60-min acclimatization period before beginning the experiment. The catheters were cleared and refilled with saline. Baseline values were determined during a half-hour interval prior to injection of the oxime, and measurements continued for 5 hr postinjection. All compounds were dissolved in sterile distilled water, and the freshly prepared solutions of 2-PAM Cl (50 μmol/kg in 1.0 ml), MMB-4 (100 μmol/kg in 1.5-2.0 ml), and ICD 467 (12.5 μmol/kg in 1.0 ml) were injected with a 1-inch 21-gauge needle in the left thigh at zero time. All compounds were obtained from Walter Reed Army Institute of Research (2-PAM Cl, WRAIR Lot No. 016411BD; MMB-4, WRAIR Lot No. 249943AF; ICD 467, WRAIR Lot No. 255737AF). To avoid additional stress on the animal, the test compound vehicle (distilled water) was injected im in the right thigh at the end of the experiment.

Myopathology. Test animals were euthanized with an intrapulmonary artery injection of T61 via the Gould catheter 2-3 days after oxime injection. Muscle biopsies from test and control sites were excised at necropsy. Samples measuring about 2 x 2 cm were placed in tissue cassettes, immersed in 10% neutral buffered formalin, and allowed at least 48 hr for fixation. Muscle samples were subsequently embedded in paraffin, cut into 5-μm sections, placed on glass slides, and stained with hematoxylin and eosin.

Cardiopulmonary measurements. Mean aortic blood pressure, pulmonary artery and pulmonary arterial wedge pressures, cardiac output, heart rate, and ECG were monitored by a Gould System 2800. Aortic and pulmonary arterial pressures were recorded continuously by attachment of vascular catheters to pressure transducers (Statham Model P50). Pulmonary arterial wedge pressures were obtained at intervals by inflating the balloon on the tip of the Gould catheter. Pulmonary artery temperature was also recorded. Pulmonary artery and pulmonary wedge pressure measurements were taken from the Gould recording at end expiration. Electrocardiograms were obtained from patch electrodes attached to the skin. Thermodilution cardiac output, using 3-ml boluses of iced 5% dextrose solution, was measured in triplicate every 30 min and calculated by a Sorenson computer, Model 41234-01. Tidal airflow was measured by a heated No. 2 Fleish pneumotachograph attached to the animal's nose cone.

Arterial blood (3.0 ml) was drawn at -30, -15, 0, 1, 2, 4, 8, 12, 16, 20, 40, 60, 80, 120, 180, 240, and 300 min postinjection. Half of each sample (1.5 ml) collected in heparinized syringes was used for the measurement of P_aO_2, P_aCO_2, and pH. The other 1.5 ml collected in syringes containing 40 μl 2% EDTA was used for the determination of microhematocrit, cholinesterase activity, and oxime concentration. Erythrocyte AChE activity was measured immediately on a Technicon Auto Analyzer II system by the method of Groff *et al.*, 1976. The method is based on the detection of thiocholine, the product of acetylthiocholine hydrolysis by cholinesterase. Precision as determined from replicate sample analyses is no more than 2% variation. The detection limit is 0.03 μM/ml/min. Concentrations of MMB-4 and 2-PAM Cl in plasma were measured with the assay of Groff and Ellin (1969). The concentration of ICD 467 was measured by the HPLC method of Shih *et al.*, 1990. Both MMB-4 and 2-PAM Cl form oximate anions following hydrolysis in an alkaline medium with detection at 375 and 336 nm, respectively. Oxime determinations may be performed on 150-μl specimens Precision

established by analyses of five replicate samples is 1.3% for MMB-4 and 1.8% for 2-PAM Cl at the 1 μg/ml concentration level. Precision at 20 μg/ml concentration level is 0.2% for both oximes. Detection limit for both oxime procedures is in the 0.1 μg/ml range.

Data analysis. Time-related plasma concentrations for the oximes were fitted to standard pharmacokinetic models using the computer program PCNONLIN (Version 3.0: Statistical Consultants, Inc., Lexington, KY). Pharmacokinetic estimates for each animal were determined from the computer program JANA (Statistical Consultants). Systemic clearance was determined on a time-weighted basis by dividing the administered dose by the area under the plasma curve. Data for model-fitting were iteratively reweighted by modulating the reciprocal of the predicted concentrations. Resolution of the appropriate model was based upon achieving a result with a minimal sum of squared residuals, the lowest possible standard deviations of parameter estimates and unbiased distribution patterns of residuals of estimates of observed *versus* predicted concentrations.

Pharmacokinetic variables obtained from the PCNONLIN program were averaged, and the averages were used to draw the best-fit line by calculating expected concentrations with small increments in time from a computerized spreadsheet (Lotus 1-2-3, Lotus Development Corp., Cambridge, MA). Data were then transferred to a computer graphics program (Sigmaplot, Jandel Scientific, Sausalito, CA) for plotting. All barred lines represent 95% confidence limits.

Cardiopulmonary data are presented as mean ± SEM. The baseline data, which served as control for each group of animals, were compared with the postinjection points. The data were analyzed by one-way ANOVA with repeated measures using BMDP program P2V (Jennrich *et al*, 1983), followed by Dunnett's test (Winer, 1971). Statistical significance was set at $p < 0.05$.

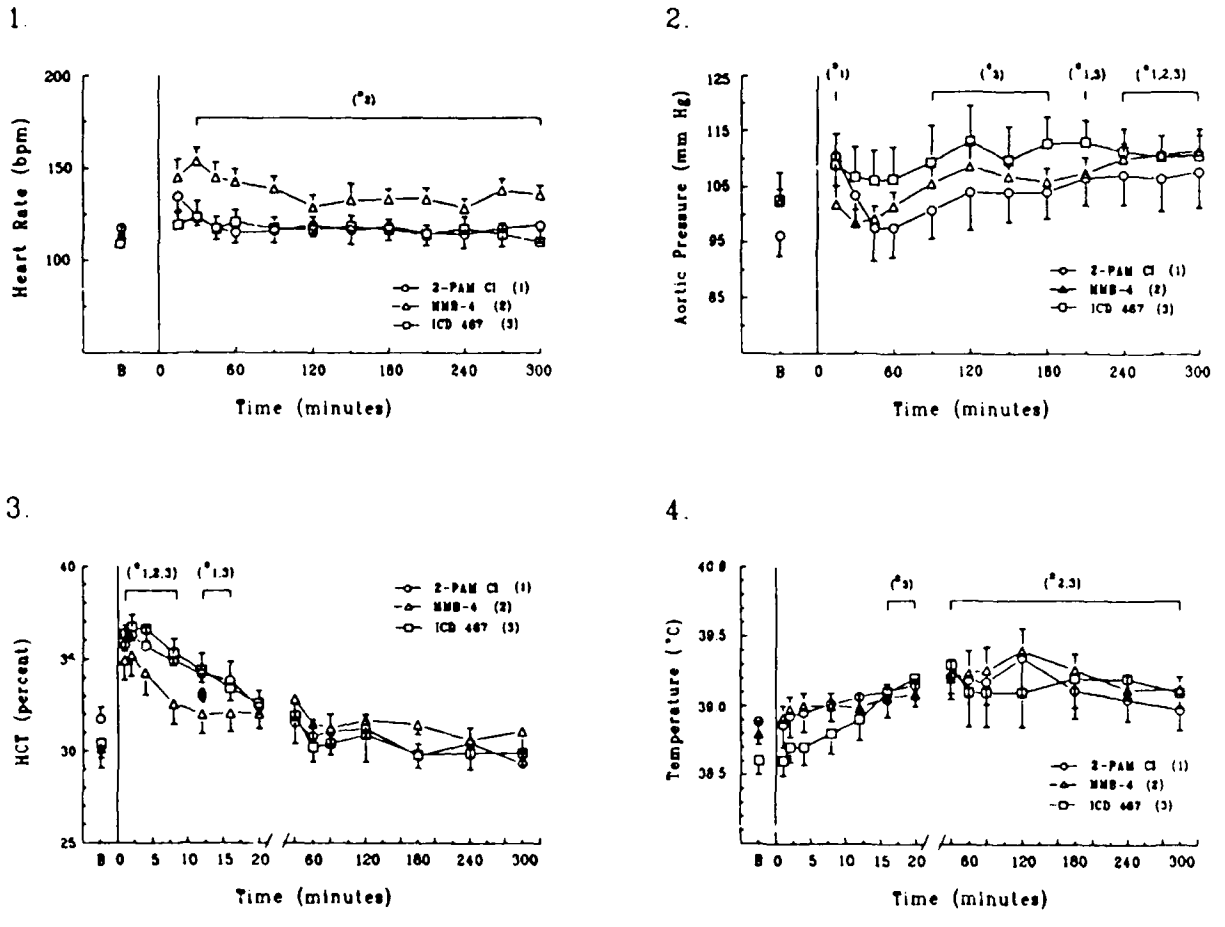
RESULTS

Clinical signs. Muscle fasciculations were observed within minutes following injection of ICD 467, but rarely after 2-PAM Cl and MMB-4, Irregular pink-purple blotches were observed on the backs of several pigs that received MMB-4.

Enzyme activity. There were insignificant changes in red blood cell AChE activity from baseline following injection of oximes.

Electrocardiograms. Most pigs, regardless of the oxime given, showed mild tachycardia and sinus arrhythmia, and increase in ventricular and/or mild to moderate supraventricular irritability after injection. These cardiac irregularities were widely and irregularly scattered throughout the 5-hr observation period. Ventricular premature beats noted in three pigs before injection, one with each compound, were increased in number following injection. Without a more definitive study it appears that the oximes may have produced some cardiac irritability or increased preexisting irritability.

Cardiovascular and temperature measurements. Figures 1, 2, 3, and 4 show the effects of the oximes on heart rate, aortic blood pressure, hematocrit, and body temperature, respectively. Heart rate increased following injection of MMB-4, but not after 2-PAM Cl or ICD 467. Except for an initial transient increase in blood pressure with 2-PAM Cl at 15 min, other significant changes were not observed until 90 minutes postinjection for ICD 467, and only during the final hours of the experiment following injection of 2-PAM Cl and MMB-4. However, with 2-PAM Cl, there was a gradual elevation of aortic pressure beginning at 45 min and continuing throughout the rest of the experiment. The hematocrit increased after injection of oximes and returned to baseline levels in less than 20 min. A sustained increase in body temperature was observed 60-300 min following injection of MMB-4 and ICD 467, but not after 2-PAM Cl. The blood gases and pH, cardiac output, pulmonary artery, and pulmonary artery wedge pressure changes were minimal after oxime administration.



Figures 1, 2, 3, 4. (1) Heart rate, (2) aortic pressure, (3) hematocrit, and (4) temperature responses respectively to im injection of oximes in pigs. Baseline values (B) were determined during a half-hour interval prior to injection of the oximes. Bars indicate ± SEM and * significant differences from baseline values for the respective numbered oximes, $p < 0.05$.

Injection site. Skeletal muscle damage at the site of injection was characterized by hemorrhage and/or edema, inflammatory cellular infiltrates (consisting primarily of macrophages with some neutrophils) with myofibril degeneration, necrosis, loss of cross striations, hyalinization, disruption of the sarcoplasm, fragmentation, and mineralization. Evidence of myofibril regeneration was also seen in some tissue sections. Such changes were observed with each oxime, in both experimental and control injection sites. Myofibril damage from MMB-4 was grossly visible, and larger by comparison to the injection sites from the other two oximes and control preparations.

Pharmacokinetics. Plasma concentration-time profiles for 2-PAM Cl in pigs are shown in Figure 5, for ICD 467 in Figure 6, and for MMB-4 in Figures 7 and 8. MMB-4 was absorbed into the blood stream approximately five times slower than ICD 467 and 2-PAM Cl at the dosages studied in pigs. The C_{max} (78 μg/ml, n=8) of MMB-4 was achieved in an average 9.1 min. The elimination of MMB-4 from the bloodstream ($\beta t_{\frac{1}{2}}$ = 158 min) was approximately two times slower than the elimination of 2-PAM Cl ($\beta t_{\frac{1}{2}}$ = 87 min); the latter is three times slower than ICD 467 ($\beta t_{\frac{1}{2}}$ = 26 min) (Table 1). The key to the pharmacokinetic parameters is contained in Appendix A, and the pharmacokinetic values for the oximes in pigs are shown in Table 1. A comparison of plasma concentration-time profiles for 2-PAM Cl in pigs and man is shown in Figure 9. In Figures 5, 6, 7, and 8, a best-fit line describing mean concentration-time data was calculated by averaging pharmacokinetics parameters obtained for each animal in the dosing group. Mean concentration-time data were not used to estimate pharmacokinetics parameters; however, they are displayed with 95% confidence intervals to represent the raw data.

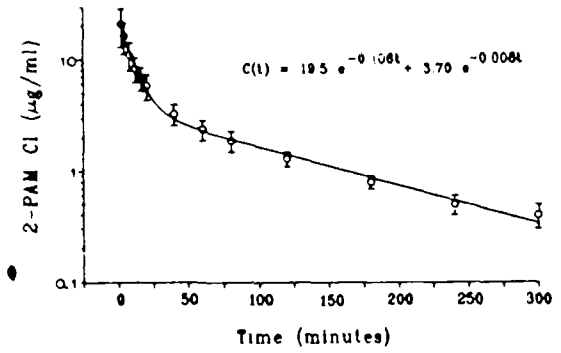


Figure 5. Concentration-time profile of 2-PAM Cl (50 μmol/kg) following im injection in pigs (n=8).

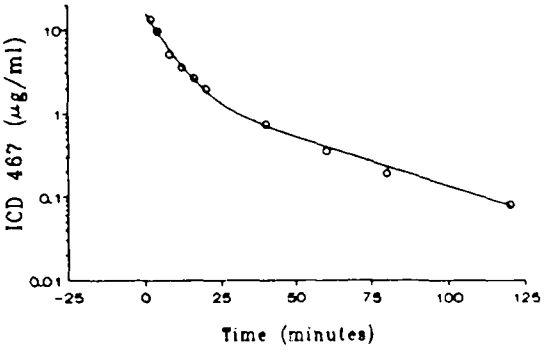


Figure 6. Concentration-time profile of ICD 467 (12.5 μmol/kg) following im injection in pigs (n=7). The confidence intervals are smaller than the symbol size and therefore are not visible on this graph.

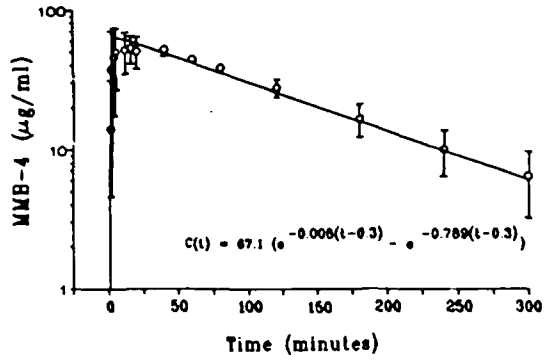


Figure 7. One-compartment concentration-time profile of MMB-4 (100 μmol/kg) following im injection in pigs (n=2).

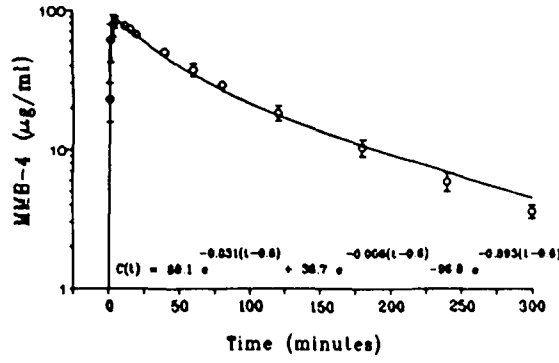


Figure 8. Two-compartment concentration-time profile of MMB-4 (100 μmol/kg) following im injection in pigs (n=6).

Model fitting. The rate of absorption of 2-PAM Cl (50.0 μmol/kg) and ICD 467 (12.5 μmol/kg) into the blood was extremely rapid and therefore could not by defined adequately by an im model. Maximum plasma concentrations (C_{max}) were achieved within 2 min of dosing, thus mimicking a bolus intravenous (iv) injection. Although both compounds were administered im, the pharmacokinetics were best described by an iv two-compartment open model with first-order distribution and elimination (Figures 5 and 6):

$$C(t) = Ae^{-\alpha t} + Be^{-\beta t}.$$

Maksimovic (1979) found a similar situation with a pharmacokinetic study of 2-PAM Cl administered im to rats. Two problems associated with not describing the absorptive kinetics (assuming 100% instantaneous absorption) were that the reported C_{max} may be over-estimated, and the volume of distribution may be underestimated (Gibaldi and Perrier, 1982). An im two-compartment open model with first-order absorption, distribution, and elimination, with a lag time, was used to describe the data in six pigs injected with MMB-4 (100 μmol/kg, im); however, the data from two pigs fit this model only marginally:

$$C(t) = Ae^{-\alpha(t-\delta)} + Be^{-\beta(t-\delta)} - (A-B)e^{-K01(t-\delta)}.$$

The data from the remaining two pigs fit an im one-compartment open model with first-order absorption and elimination, with a lag time:

$$C(t) = \frac{D}{V_d}\frac{K01}{K01 - K10}\left(e^{-K10(t-\delta)} - e^{-K01(t-\delta)}\right).$$

The major problem in describing the pharmacokinetics for the latter two animals was that neither exhibited a distinct distribution phase. Standard errors associated with the distribution phase were extremely large in each case when the data were fit to the two-compartment model. Therefore, the one-compartment model was used. Plasma concentration-time profiles for MMB-4 in pigs fitting the one-compartment model and the two-compartment model are shown in Figures 7 and 8, respectively.

TABLE 1. Pharmacokinetic Parameter Values For 2-PAM Cl, ICD 467 and MMB-4 Intramuscularly Administered to Swine

	2-PAM Cl [two-compartment] (50.0 μmol/kg) or (8.6 mg/kg) (n = 8)	ICD 467 [two-compartment] (12.5 μmol/kg) or (3.8 mg/kg) (n = 7)	MMB-4 [two-compartment] (100.0 μmol/kg) or (32.9 mg/kg) (n = 6)	MMB-4 [one-compartment] (100.0 μmol/kg) or (32.9 mg/kg) (n = 2)
Parameter[a]	Value (± SD)	Value (± SD)	Value (± SD)	Value (± SD)
A	19.5 (±8.6)	14.1 (±3.4)	60.2 (±16.8)	[b]
B	3.7 (±1.3)	2.0 (±0.8)	36.7 (±25.1)	[b]
α	0.106 (±0.038)	0.153 (±0.035)	0.031 (±0.017)	[b]
β	0.008 (±0.001)	0.027 (±0.005)	0.006 (±0.004)	[b]
K01	[b]	[b]	0.893 (±0.638)	0.789 (±0.997)
K10	0.036 (±0.010)	0.098 (±0.019)	0.014 (±0.002)	0.008 (±0.001)
K12	0.054 (±0.027)	0.040 (±0.015)	0.009 (±0.005)	[b]
K21	0.025 (±0.007)	0.043 (±0.011)	0.017 (±0.014)	[b]
AUC	632 (±138)	164 (±14)	7,020 (±361)	8,749 (±552)
C_l	14.2 (±3.1)	23.6 (±1.9)	4.7 (±0.2)	3.8 (±0.2)
V_d	433 (±163)	253 (±67)	360 (±46)	496 (±42)
K01$t_{1/2}$	[b]	[b]	1.0 (±0.5)	4.4 (±5.4)
K10$t_{1/2}$	21.0 (±6.6)	7.4 (±1.8)	53.3 (±8.7)	91.1 (±14.3)
$\alpha t_{1/2}$	7.1 (±2.1)	4.7 (±1.1)	29.8 (±16.6)	[b]
$\beta t_{1/2}$	86.8 (±14.7)	26.3 (±5.9)	157.6 (±107.9)	[b]
C_{max}	21.0 (±8.8)[c]	13.4 (±2.8)[c]	83.8 (±12.4)	59.4 (±13.1)
T_{max}	[d]	[d]	5.9 (±1.7)	18.3 (±19.9)
δ	[d]	[d]	0.6 (±0.3)	0.4 (±0.5)
Sum sq. resid.	23.4 (±42.6)	4.38 (±3.0)	35.7 (±10.9)	85.9 (±70.1)
r of y, yhat	0.989 (±0.014)	0.994 (±0.003)	0.999 (±0.001)	0.991 (±0.010)

[a] Appropriate units for each parameter are defined in Appendix A.
[b] Parameter is not defined by this model.
[c] This value was obtained directly from the raw plasma concentration-time data.
[d] An iv model assumes C_{max} to be instantaneous; therefore, T_{max} and δ will always be equal to zero.

DISCUSSION

A few cardiovascular changes were observed which were statistically significant at the dosages of oximes used in these experiments. The transient increases in hematocrit with all oximes were probably due to splenic contraction associated with the pigs' response to needle puncture and, perhaps, to injection (Figure 1). Pain at the injection

site has been reported in human subjects following im injection of 2-PAM Cl (Vojvodic and Boskovic, 1976; Sidell and Groff, 1971).

Although aortic pressure was increased 3.5-5.0 hr after injection of 2-PAM Cl, intra-arterial (ia) injections in another study in pigs with larger doses of 2-PAM Cl (116 μmol/kg ia) did not alter blood pressure or heart rate (Wade *et al*, 1988). Large doses of 2-PAM Cl given orally, iv, and im have been reported to be without effect on blood pressure and heart rate in humans (Swartz and Sidell, 1974; Sidell and Groff, 1971; Calesnick *et al*, 1967; Vojvodic and Boskovic, 1976).

Elevated blood pressure peaks at 2 min were reported following iv injections of 5 to 40 mg/kg 2-PAM Cl in open chest anesthetized dogs (Barnes *et al*, 1972). The results of their study suggested a direct stimulatory action of 2-PAM Cl on the heart and vascular smooth muscle. In comparison, the arterial pressures in pigs receiving 2-PAM Cl or MMB-4 increased gradually with time and reached maximal levels in the final hours of the experimental period. In pigs the blood concentrations of oximes were maximal within minutes of injection and decreased rapidly with time. As a result, the maximal pressure changes do not correlate well with maximal blood concentrations of oximes. A parallel control study utilizing pigs not injected with oximes might have indicated whether the pressure and other physiological changes observed were influenced by psychological stress associated with the procedures.

Little inhibition of red blood cell AChE was observed following administration of ICD 467 in pigs. However, with this oxime in rabbits, moderate inhibition of whole blood AChE was reported (Harris *et al*, 1990). The opposing results are presumably due to differences in the assay procedures employed. In *in vitro* studies, Harris *et al* (1990) showed that washing of red blood cells resulted in loss of ICD 467 prior to determination of AChE. The initial dilution of the sample with our assay may also result in loss of ICD 467 thus eliminating the effect on AChE.

Wade *et al* (1988) also measured the plasma 2-PAM Cl levels in normal and hemorrhaged pigs. Although the study was not designed to investigate the pharmacokinetics of 2-PAM Cl, they reported a half-time elimination ($\beta t_{\frac{1}{2}}$) of 65-75 min (n=7). Concentration-time profile comparisons between these two studies are depicted in Figure 9.

Green *et al* (1986) described im pharmacokinetics of 2-PAM Cl (116, 233, and 466 μmol/kg) in rats with a one-compartment model. They observed absorptive kinetics that were much slower than our results. Their reported maximum concentrations (C_{max}) for rats were observed approximately 15 min after dosing, whereas in pigs, C_{max} was 0 min.

Sidell and Groff (1971) described the pharmacokinetics of 2-PAM Cl in man after iv and im injections. A comparison of concentration-time profiles between pigs (our study: 50 μmol/kg im) and humans (58 μmol/kg iv) indicates similar elimination kinetics (Figure 9). In man, $\beta t_{\frac{1}{2}}$ of 2-PAM Cl was 79 min, (Sidell and Groff, 1971). With pigs, we have determined $\beta t_{\frac{1}{2}}$ of 2-PAM Cl to be 87 min. Rate constants (β) describing the elimination of 2-PAM Cl from both pigs and humans were virtually identical.

Humans and pigs differed considerably in their absorption and elimination kinetics when 2-PAM Cl was administered by an im route. The C_{max} of 2-PAM Cl (50 μmol/kg im) in pigs occurred almost instantaneously, while in humans (58 μmol/kg im) it occurred approximately 20 min after injection. Therapeutic plasma levels (4 μg/ml) (Sundwall, 1961) were achieved, however, within 5-10 min and were sustained for almost 1 hr after dosing. Although the apparent instantaneous rate of absorption for ICD 467

and 2-PAM Cl were similar, the half-times of elimination were different, being more rapid with ICD 467. To our knowledge, no other pharmacokinetic studies have been published on ICD 467 to date.

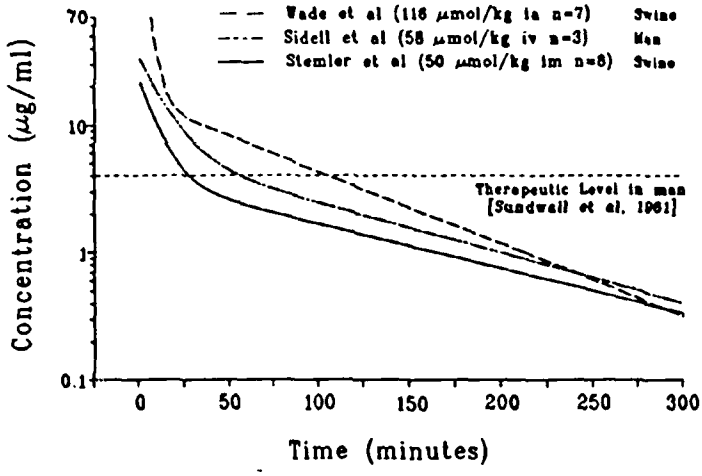


Figure 9. Concentration-time profile of 2-PAM Cl: Comparison between pigs and humans. The pharmacokinetics for all studies other than ours were reprocessed according to our method of analysis by directly model-fitting the respective mean concentration-time data. Raw data and 95% confidence intervals are not displayed for purposes of graphical clarity.

A pharmacokinetic study of MMB-4 (50 μmol/kg im) in rabbits (Woodard and Lukey, 1991) at half our MMB-4 dose revealed minor interspecies variances in metabolic rates. The pharmacokinetics of MMB-4 (50 μmol/kg im) in rabbits were described with a one-compartment open model with first-order absorption and elimination without a lag time. We found that pigs' im absorption differs from that of rabbits by a slight lag time. The lag time may be due to physiological differences in blood flow at the site of injection in the pig and the rabbit. MMB-4 was generally absorbed two times faster into the blood stream in pigs (9.1 min) than in rabbits (15.7 min). The slower elimination rate for MMB-4 is probably metabolic in nature, since the compound has poor lipophilic properties. There was no distributive phase observed in rabbits. Pharmacokinetics of MMB-4 in species other than the rabbit have not been reported in the literature.

The effectiveness of 4-carboxamidopyridinium (1)methyl-(2"hydroxyiminomethyl-pyridinium (1")methyl)ether dichloride (HI-6), MMB-4 and 2-PAM Cl as reactivators of soman-inhibited AChE was compared in rabbits (Harris *et al*, 1990). Treatment with HI-6 and MMB-4, but not 2-PAM Cl, resulted in significant reactivation of unaged soman-inhibited whole blood AChE and diaphragm cholinesterase, but not brain AChE. Moreover, Koplovitz and Stewart (1989) reported that treatment with adequate amounts of atropine plus HI-6 or MMB-4 was superior to 2-PAM Cl, when equivalent atropine therapy was simultaneously administered against soman intoxication in rabbits.

In conclusion, significant blood pressure effects were noted after all oximes, but they were not of serious consequence and occurred near the end of the experiments. There was an elevation in heart rate after MMB-4, which was still within physiological limits. 2-PAM Cl and ICD 467 were rapidly absorbed. ICD 467 was distributed slightly faster than 2-PAM Cl and had a faster elimination rate. MMB-4 showed a slight lag time before detectable levels were observed in the blood; thus, MMB-4 may not reach the same plasma levels as 2-PAM Cl in an equal period of time. However, MMB-4 is retained in the blood stream longer than 2-PAM Cl, and may have some potential for extending therapeutic effects. A combination of MMB-4 plus a rapidly absorbed oxime should be investigated for improved efficacy. The comparison of 2-PAM Cl (im) in several species suggests that the absorptive phase in pigs differs from that seen in man. However, the elimination rates for 2-PAM Cl are comparable.

REFERENCES

Banakar, U.V. and Patel, U.N. (1988). Pralidoxime Chloride. In *Analytical Profiles of Drug Substances* (Florey, Klaus, Ed.), Vol 17, pp. 533-569. Academic Press, New York.

Barnes, G.E., Bishop, V.S. and Kardon, M.B. (1972). Cardiac and peripheral vascular effects of pralidoxime chloride. *Eur. J. Pharmacol.* **20**, 188-194.

Calesnick, B., Christensen, J.A., and Richter, M. (1967). Human toxicity of various enzymes. *Arch. Environ. Health.* **15**, 599-608.

Fleisher, J.H., and Harris, L.W. (1965). Dealkylation as a mechanism for aging of cholinesterase after poisoning with pinacolyl methylphosphonofluoridate. *Biochem. Pharmacol.* **14**, 641-650.

Fleisher, J.H., Harris, L.W., Miller, G.R., Thomas, N.C., and Cliff, W.J. (1970). Antagonism of sarin poisoning in rats and guinea pigs by atropine, oximes, and mecamylamine. *Toxicol. Appl. Pharmacol.* **16**, 40-47.

Fleisher, J.H., Harris, L.W., and Murtha, E.F. (1967). Reactivation by pyridinium aldoxime methochloride (PAM) of inhibited cholinesterase activity in dogs after poisoning with pinacolyl methylphosphonofluoridate (soman). *J. Pharmacol. Exp. Ther.* **156**, 345-351.

Gibaldi, M., and Perrier, D. (1982). *Pharmacokinetics.* Dekker, New York.

Green, M.D., Talbot, B.G., and Clark, C.R. (1986). Pharmacokinetics of pralidoxime chloride in the rat. *Life Sci.* **39**, 2263-2269.

Groff, W.A., and Ellin, R.I. (1969). A new and rapid determination of pyridinium aldoximes in blood and urine. *Clin. Chem.* **15**, 72-83.

Groff, W.A., Kaminskis, A., and Ellin, R.I. (1976). Interconversion of cholinesterase enzyme activity units by the manual ΔpH method and a recommended automated method. *Clin. Toxicol.* **9**, 353-358.

Harris, L.W., Anderson, D.R., Lennox, W.J., Woodard, C.L., Pastelak, A.M. and Vanderpool, B.A. (1990). Evaluation of several oximes as reactivators of unaged soman-inhibited whole blood acetylcholinesterase in rabbits. *Biochem. Pharmacol.* **40**, 2677-2682.

Harris, L.W., Fleisher, J.H., Clark, J. and Cliff, W.J. (1966). Dealkylation and loss of capacity for reactivation of cholinesterase inhibited by sarin. *Science.* **154**, 404-407.

Harris, L.W., Heyl, W.C., Stitcher, D.L., and Broomfield, C.A. (1978). Effects of 1,1'oxydimethylene bis-(4-tert-butylpyridinium chloride) (SAD-128) and decamethonium on reactivation of soman- and sarin-inhibited cholinesterase by oximes. *Biochem. Pharmacol.* 27, 757-761.

Jennrich, R., Sampson, P., and Frane, J. (1983). BMDP program P2V, analysis of variance and covariance including repeated measures. In *BMDP Statistical Software* (W.L. Dixon, Chief Ed.), pp. 359-387. Univ. of California Press, Berkeley, CA.

Koplovitz, I., and Stewart, J.R. (1988). Novel imidazolium oximes as improved nerve agent antidotes. *USAMRICD-TR-88-06* (AD# A198242), US Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD.

Koplovitz, I., and Stewart, J.R. (1989). Efficacy of oximes combined with atropine against soman poisoning in the atropinesterase-free rabbit. In *Proceedings of the 1989 Medical Defense Bioscience Review. DTIC-B139550.* U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD.

Maksimovic, M. (1979). Oximes HI-6 and PAM chloride; comparative pharmacokinetic studies after intramuscular and oral administration in the rat. *Arh. Hig. Rada Toksikol.* 30, 227-239.

Panepinto, L.M. (1986). Laboratory methodology and management of swine in biomedical research. In *Swine in Biomedical Research* (Tumbleson, M.E., Ed.) Vol I, pp. 97-109. Plenum, New York.

Phillips, R.W., and Tumbleson, M.E. (1986). Models. In *Swine in Biomedical Research* (Tumbleson, M.E., Ed.), Vol. I, pp. 437-440. Plenum, New York.

Shih, M.L., Smith, J.R., and Salem, G.W. (1990) High-performance liquid chromatographic analysis of imidazolium and pyridinium oximes in plasma and urine. *J. Chromatog.* 527, 371-379.

Sidell F.R., and Groff, W.A. (1971). Intramuscular and intravenous administration of small doses of 2-pyridinium aldoxime methochloride to man. *J. Pharm. Sci.* 60, 1224-1228.

Sundwall, A. (1961). Minimum concentrations of *n*-methylpyridinium-2-aldoxime methane sulphonate (P2S) which reverse neuromuscular block. *Biochem. Pharmacol.* 8, 413-421.

Swartz, R.D., and Sidell, F.R. (1974). Renal tubular secretion of pralidoxime in man. *Proc. Soc. Exp. Biol. Med.* 146, 419-424.

Talbot, B.G., Anderson, D.R., Harris, L.W., Yarbrough, L.W. and Lennox, W.J. (1988). A comparison of *in vivo* and *in vitro* rates of aging of soman-inhibited erythrocyte acetylcholinesterase in different animal species. *Drug Chem. Toxicol.* 11, 289-305.

Vojvodic, V. and Boskovic, B. (1976). *A Comparative Study of Pralidoxime, Obidoxime and Trimedoxime in Healthy Men Volunteers and in Rats.* Almqvist & Wiksell (Stockholm International Peace Research Institute (SIPRI)).

Wade, C.E., Waring, P.P., Trail, D.S., Williams, B.F., Bonner, G.D., and Gildengorin, V.L. (1988). Effects of atropine, 2-PAM or pyridostigmine in euvolemic or hemorrhagic conscious swine. *Mil. Med.* **153**, 470-476.

Winer, B. J. (1971). *Statistical Principles in Experimental Design.* 2nd ed., pp. 201-203. McGraw-Hill, New York.

Woodard, C.L., and Lukey, B.J. (1991). MMB-4 Pharmacokinetics in rabbits after intravenous and intramuscular administration. *Drug Metabolism and Disposition* **19**, 283-284.

APPENDIX A. Pharmacokinetic Parameters

C	=	plasma concentration (μg/ml)
t	=	time (min)
D	=	administered dose (μg/kg)
A	=	coefficient constant for distribution (μg/ml)
B	=	coefficient constant for elimination (μg/ml)
α	=	rate constant of the distribution phase (min^{-1})
β	=	rate constant of the elimination phase (min^{-1})
$K01$	=	rate constant of absorption (min^{-1})
$K10$	=	overall rate constant of elimination (min^{-1})
$K12$	=	rate constant of transfer from central compartment to peripheral compartment (min^{-1})
$K21$	=	rate constant of transfer from peripheral compartment to central compartment (min^{-1})
AUC	=	area under plasma concentration-time curve (μg/ml/min)
C_l	=	systemic plasma clearance (ml/min/kg)
V_d	=	apparent volume of distribution (ml/kg)
$K01t_{\frac{1}{2}}$	=	half-time of absorptive phase (min)
$K10t_{\frac{1}{2}}$	=	overall half-time of elimination (min)
$\alpha t_{\frac{1}{2}}$	=	half-time of distribution phase (min)
$\beta t_{\frac{1}{2}}$	=	half-time of elimination phase (min)
C_{max}	=	maximum plasma concentration (μg/ml)
T_{max}	=	time to reach maximum plasma concentration (min)
δ	=	lag time for drug absorption into central compartment (min)