Defence Research and Development Canada
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Chemical Weapon Agent Studies in Domestic Swine:

Part 5: Comparative Bioavailability of Different HI-6 Salt Forms in the Domestic Pig

Prepared By:
Kinchyle Enterprises Inc.
Medicine Hat
Alberta, Canada
PWGSC Contract Number: W7702-99-R785

Defence R&D Canada
Contract Report
DRDC Suffield CR 2002-170
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IMPORTANT INFORMATIVE STATEMENTS

The scientific or technical validity of this Contract Report is entirely the responsibility of the Contractor and the contents do not necessarily have the approval or endorsement of the Department of National Defence of Canada.

In conducting this research, the contractor adhered to the "Guidelines to the Care and Use of experimental Animals" and" Ethics of Animal Experimentation" published by the Canadian Council on Animal Care. The animal care committee at DRDC Suffield approved the relevant protocol (TWS01-01) prior to experimentation.

Abstract

A well-developed anesthetized domestic swine model has been utilized to study the following effect of chemical weapons (CW):

01- Comparative bioavailability of different HI-6 salt forms in the domestic pig
The bioavailability of the dihydrochloride salt of HI-6a (currently utilized) was compared to that of the dimethanesulfonate salt (HI-6b). The latter is more water-soluble at low temperatures. The salts appear similarly clinically, biochemically or hematologically in the "equivalent" doses administered (HI-6 dihychloride = 500mg, HI-6 dimethanesulfonate = 633mg.) A notable exception might be the observation that the combination of pyridostigmine (PYR)/HI-6b increases airway resistance. Timed plasma and urine samples were collected for HPLC analysis.

Résumé

Nous avons utilisé un modèle bien établi de porc domestique anesthésié pour étudier les effets ci dessous d'armes chimiques (CW) :

01- Biodisponibilité comparative de différentes formes salines de HI-6 présentes dans un porc domestique.
Nous avons comparé la biodisponibilité du sel de dichlorhydrate de HI-6a (actuellement utilisé) à celle du sel de diméthanesulfonate (HI-6b), qui est plus hydrosoluble à une basse température. Les sels semblent similaires sur le plan clinique, biochimique ou hématologique d'après les doses « équivalentes » administrées (dichlorhydrate de HI-6a = 500 mg et diméthanesulfonate HI-6b = 633 mg). La seule exception notable pourrait être l'observation selon laquelle la combinaison de pyridostigmine et de HI-6b augmente la résistance des voies aériennes. Des échantillons de plasma et d'urine ont été recueillis par intervalles aux fins d'analyse par CLHP.

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(CWDSMp5.)
(02/09/16)

Pt.5: Comparative Bioavailability of Different HI-6 Salt Forms in the Domestic Pig

Submitted: September 26th, 2002

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Executive Summary

A well-developed anesthetized domestic swine model has been utilized to study the following effect of chemical weapons (CW):

01- comparative bioavailability of different HI-6 salt forms in the domestic pig

The bioavailability of the dihydrochloride salt of HI-6a (currently utilized) was compared to that of the dimethanesulfonate salt (HI-6b). The latter is more water-soluble at low temperatures. The salts appear similar clinically, biochemically or hematologically in the "equivalent" doses administered (HI-6 dihydrochloride = 500 mg, HI-6 dimethanesulfonate = 633 mg). A notable exception might be the observation that the combination of pyridostigmine (PYR)/HI-6b increases airway resistance.

Dr. MG Hamilton collected timed plasma and urine samples for HPLC analysis.

Introduction

Therapies against organophosphate (OP) nerve agent poisoning include the prophylactic administration of a reversible anticholinesterase like pyridostigmine and treatment with an anticholinergic like atropine plus an oxime acetylcholinesterase i.e. HI-6. Because the dimethanesulfonate salt of HI-6 is more water-soluble at low temperatures, its bioavailability was compared to that of the dihydrochloride salt of HI-6 (currently utilized).

Because of its many anatomical, physiological and biochemical similarities to humans the domestic pig has been extensively used as an animal model for various human physiological and pathophysiological states [1,2]. This is also true with respect to CW studies.

In particular, a domestic swine model (DSM) is utilized to evaluate the effects of anticholinergics, oximes [3-5] and anticholinesterase drugs [3,6,7].

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Methods

A general methodology for all parts of this contract follows. Deviations from the general methodology are noted in either the special methodology section or the results section.

Animals:

147 10 week-old Yorkshire-Landrace cross barrows (*Sus scrofa domestica*) were obtained from a local commercial supplier who has supplied 600+ animals of consistent quality to DRDC-Suffield over the past 5 years. This herd has been tested for the ryanodine receptor mutation associated with malignant hyperthermia syndrome (MHS) and found to be not susceptible [1]. This age group/sex/size were chosen originally because of prior work in this field by other investigators, ease of handling, technical considerations (anesthesia/surgical) and ready applicability of many commercially available/applicable medical devices.

Animals were housed indoors in groups of three at the vivarium complex at DRDC Suffield. Animals were acclimatized prior to use with a 12-hour light cycle at 20 °C. Animals received 16% hog grower (United Grain Growers Limited, Okotoks, Alberta, Canada) and were permitted tap water *ad libitum*. The mean body weight at the time of surgery was 20.2 kg ± 1.46 (mean ± SD, n=147). In conducting this research, the contractor adhered to the "Guide to the Care and Use of Experimental Animals" and "The Ethics of Animal Experimentation" published by the Canadian Council on Animal Care. The animal care committee at DRDC Suffield prior to experimentation approved protocols.

Animals were fed until the evening prior to surgery and allowed tap water *ad libitum* until the time of the experiment. The animals were unpremedicated. After weighing, all animals underwent an inhalation induction in the transport container. Isoflurane 5 % (Abbott Laboratories, Montreal, Quebec, Canada) in a carrier gas of oxygen (O_2) at a flow rate of 8 $L.min^{-1}$ was administered utilizing a Boyle anesthesia machine with a circle patient anesthesia circuit. Post-induction, the animal was placed in the dorsal recumbent position on a heated operating table. Animals were intubated with a 6.5 mm internal diameter (ID) cuffed oral endotracheal tube (Ruschelit, Willy Rusch AG, 71394 Kernen, Germany) and the cuff was inflated with room air to achieve a seal. The isoflurane concentration was reduced to 3% in 100% O_2 at a flow rate of 1 $L.min^{-1}$. The animals received intravenous fluids via a 22-gauge catheter. A 20-gauge IV catheter was inserted into the right femoral artery for blood pressure measurement and blood sampling. All catheters

were fixed to the skin, and all incisions closed. Core body temperature was maintained at approximately 38.5 °C with a Sage-London Industries Inc. Therm-o-matic™ heated operating table. The isoflurane concentration was reduced to 2% in 100% O_2 at a flow rate of 1 $L.min^{-1}$. Blood samples were drawn after a 30 min equilibration period on 2% isoflurane (steady-state anesthesia =SSA) to establish baseline anesthetic ChE values. This level of anesthesia is approximately 1 minimum alveolar concentration (MAC) for isoflurane/domestic swine [2-4]. At the same time all animals received iv normal saline (sodium chloride 0.9%, Abbott Laboratories Ltd.) Animals in the HI-6 study received iv normal saline at a rate of 7.74 $mL.kg^{-1}.h^{-1}$ ± 0.66 (mean ± SD, n=44) via a volumetric infusion pump (Travenol FloGard 8000, Travenol Laboratories). Urinary output was measured at 3.54 $mL.kg^{-1}.h^{-1}$ ± 1.56 (mean ± SD, n=147 by a percutaneously placed needle cystostomy.

Monitors:

A capnographic sensor to measure end-tidal partial pressure of carbon dioxide in mm Hg ($P_{ET}CO_2$) and a pulse oximeter to measure percentage oxygen saturation of arterial blood (SpO2) were connected to a multifunction monitor (Nellcor N1000, Nellcor Inc., Hayward, CA) which was in turn connected via analog outputs to a Coulbourn Instruments high speed videograph I/O port (L19-02, Coulbourn Instruments, Inc., Allentown, PA). Non-respiratory physiological measurements included rectal temperature (°C), heart rate (HR: bpm), mean arterial pressure (MAP: mmHg), expired CO_2 ($P_{ET}CO_2$: kPa and simple electroencephalography (EEG). Data was collected with a Coulbourn Instruments LabLinc S computer interface (Coulbourn Instruments, Inc., Allentown, PA) to a Dell Optiplex 590 IBM compatible PC. Data was displayed and stored using WinGraph for Windows software.

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 was displayed and stored using custom programmed software (Pulmonary Monitor 1.01, Black Cat Software, Calgary, Alberta, Canada). Respiratory parameters included respiratory frequency (f: breathes per min), tidal volume (V_T: mL), minute ventilation (V_E: mL per min), peak inspiratory flow rate (PIFR), peak expiratory flow rate (PEFR), airway resistance (R_{aw}), dynamic compliance (C_{ody}), work of breathing (WoB), and mouth occlusion pressure ($P_{0.1s}$).

After the monitors were placed, the animals were stabilized for at least 30 min, during which time steady-state anesthesia (SSA) was established. Baseline physiological and biochemical values were obtained prior to drug administration; measurements were continued for the duration of anesthesia and displayed on a standard monitor.

Hematology, Biochemistry, Cholinesterase (ChE) Activity:

Blood for hematology, biochemistry and electrolytes was obtained prior to induction of general anesthesia (GA), after the equilibration period of 30 minutes on 2% isoflurane and 1, 3 and 5 hours post 30 minute equilibration. Analysis was performed using IDEXX Vetlab blood chemistry analyzer, hematology analyzer and electrolyte analyzer (IDEXX Laboratories, Inc., Westbrook, Maine).

The following biochemical parameters could be examined:

TP	total protein
Alb	albumen
Glob	globulin
Chol	cholesterol
Trig	triglycerides
Glu	glucose
Ca^{++}	calcium
Mg^{++}	magnesium
Phos	phosphate
TBil	total bilirubin
AST	aspartate aminotransferase (SGOT)
ALT	alanine aminotransferase (SGPT)
GGT	gamma-glutamyltransferase
AlkP	alkaline phosphatase
LDH	lactate dehydrogenase
CK	creatine kinase
Amyl	amylase
Lipa	lipase
Crea	creatinine
Urea/BUN	urea/blood urea nitrogen

The following electrolyte parameters could be examined:

K^{+}	potassium
Na^{+}	sodium
Cl^{-}	chloride

The following hematological parameters could be examined:

Hct	hematocrit
Hgb	hemoglobin
MCHC	mean corpuscular hemoglobin concentration
WBC	white blood cell
Gran	absolute granulocytes
% Gran	% granulocytes

L/M	absolute lymphocytes/monocytes
% L/M	% lymphocytes/monocytes
Eos	eosinophils
PLT	platelets
Retics	reticulocytes

Blood for ChE activity was obtained pre-induction, after the establishment of steady state anesthesia (SSA) and at 15 minute intervals during the 6 hour experimental period [5].

Arterial blood gas (ABG) analysis was be undertaken following the establishment of SSA and at hours 1, 3 and 5 of the experimental period using a Radiometer ABL5 arterial blood gas analyzer (London Scientific Limited, London, ON, Canada).

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2. Lundeen G, Manohar M, Parks C. Systemic distribution of blood flow in swine while awake and during 1.0 and 1.5 MAC isoflurane anesthesia with and without 50% nitrous oxide. Anesth Analg 1983; 62:499-512.
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4. Eger EI, Johnson BH, Weiskopf RB et al. Minimum alveolar concentration of I-653 and isoflurane in pigs: Definition of a supramaximal stimulus. Anesth Analg 1988: 67:1174-1176.
5. Johnson CD, Russell RL: A rapid, simple radiometric assay for cholinesterase, suitable for multiple determinations. Analyt. Biochem 1975; 64:229-238.

Specific Methodology

Experiment	HI-6 bioavailability
Anesthesia	isoflurane/oxygen (2%/1L/min)
Monitors	Nellcor/Bicore, EKG, EEG, Temperature, Arterial line, IV Cardiomax
Endpoint	follow 6 hours after HI-6 application
Blood sampling	IDEXX: awake and at 5 hrs after HI-6 AChE: awake, SSA and 15 min intervals for 1 h (2 h in pyridostigmine (PYR) groups), then 30 min for 5 h after drug application till 6 h point ABL 5: SSA, 1, 3 and 5 h after HI-6, (+ 1 h after PYR in PYR groups) HPLC: 1, 3, 5, 10, 15, 30, 45, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330, 360 min after HI-6
Urine sampling	control (after catheter) and 1, 3 and 5 h after HI-6 (chemistry) For HPLC as blood samples (exclude 1, 3, 5 and 10 min)
Special	two different HI-6 salts are studied with 4 drug regimens each: 1) HI-6 alone 2) HI-6 and atropine (2 mg) 3) PYR / HI-6 4) PYR/ HI-6 and atropine (2 mg)

HI-6a = HI-6 dichloride (500 mg to be given)
HI-6b = HI-6 dimethylsulfonate (633 mg to be given as equivalent dose)

Animal reference Numbers

397-420 (corrected: 397-436, + 465-470)

16 pigs were amended: 8 to increase group size from 3 to 4.

4 pigs were added to be control pigs, as we did not know the baseline cardiac output values during anesthesia.

4 pigs were added to follow the development of ChE – levels through 6 h of anesthetic after the administration of PYR 1 mg/kg.

After initial review of data, 6 pigs where added to achieve group sizes of four (because of wrong drug dosage in two cases, and because of IDEXX machinery malfunction twice over the course of the experiments).

Results

Cholinesterase (ChE) activity

Figure1-% ChE Activity in all non-PYR HI-6 groups compared to controls

Figure 2-% ChE Activity in HI-6a / HI-6b compared to controls, Mean and SD

Figure 3-% ChE Activity in HI-6a / HI-6b with atropine, compared to controls, Mean and SD

Figure 4-% ChE Activity in all PYR-HI-6 groups, compared to controls and PYR only

Figure 5-% ChE Activity in HI-6a / HI-6b compared to controls and PYR only, Mean and SD

Figure 6-% ChE Activity in HI-6a / HI-6b with atropine, compared to controls and PYR only, Mean and SD

Results

Arterial Blood Gases (ABG)

Table 1-Arterial blood gases in the HI-6 groups at SSA compared to controls

		pH	pCO_2 (mmHg)	pO_2 (mmHg)	AaDpO2 (mmHg)	HCO^{3-} (mmol/L)	ABE (mmol/L)	SBE (mmol/L)	SBC (mmol/L)	tCO2 (Vol%)

Controls	n=5	7.37	50	385	219	28	3	3	27	66
HI-6a	n=3	7.37	58	396	209	32	6	7	30	75
HI-6b	n=4	7.35	60	339	266	32	6	7	30	75
HI-6a / Atropine	n=4	7.36	57	443	163	31	5	5	29	72
HI-6b / Atropine	n=3	7.38	52	485	126	29	4	5	28	69
PYR / HI-6a	n=4	7.38	52	319	286	30	4	5	28	69
PYR / HI-6b	n=4	7.36	59	410	188	32	6	7	30	76
PYR / HI-6a / Atropine	n=5	7.38	52	449	154	30	5	5	29	70
PYR / HI-6b / Atropine	n=6	7.36	54	353	246	29	4	4	28	68
PYR	n=4	7.36	58	413	190	31	6	6	30	73

Table 2-Arterial blood gases in the PYR groups1 h post PYR, pre HI-6

		pH	pCO_2 (mmHg)	pO_2 (mmHg)	AaDpO2 (mmHg)	HCO^{3-} (mmol/L)	ABE (mmol/L)	SBE (mmol/L)	SBC (mmol/L)	tCO2 (Vol%)
PYR / HI-6a	n=4	7.39	53	221	394	31	5	6	29	72

PYR / HI-6b	n=4	7.37	60	196	407	33	7	8	31	78
PYR / HI-6a / Atropine	n=5	7.43	46	343	269	29	5	5	29	68
PYR / HI-6b / Atropine	n=6	7.40	51	390	208	29	5	5	29	68
PYR	n=4	7.39	52	311	299	30	5	6	29	70

Table 3-Arterial blood gases in the HI-6 groups, 1 h post HI-6

		pH	pCO_2 (mmHg)	pO_2 (mmHg)	AaDpO2 (mmHg)	HCO^{3-} (mmol/L)	ABE (mmol/L)	SBE (mmol/L)	SBC (mmol/L)	tCO2 (Vol%)
Controls	n=5	7.44	42	335	278	28	4	4	28	64
HI-6a	n=3	7.41	52	424	188	32	7	7	31	75*
HI-6b	n=4	7.41	52	338	275	32	7	7	31	75**
HI-6a / Atropine	n=4	7.39	54	346	264	31	6	7	30	74**
HI-6b / Atropine	n=3	7.44	45	352	267	30	6	6	30	69
PYR / HI-6a	n=4	7.43	46	276	337	29	5	5	29	68
PYR / HI-6b	n=4	7.43	48	333	276	30	6	6	30	71
PYR / HI-6a / Atropine	n=5	7.42	48	367	241	30	6	6	30	70
PYR / HI6-b / Atropine	n=6	7.40	51	353	249	30	5	5	29	70
PYR	n=4	7.40	50	222	390	30	5	5	29	70

Table 4-Arterial blood gases in the HI-6 groups, 3 h post HI-6

		pH	pCO_2 (mmHg)	pO_2 (mmHg)	AaDpO2 (mmHg)	HCO^{3-} (mmol/L)	ABE (mmol/L)	SBE (mmol/L)	SBC (mmol/L)	tCO2 (Vol%)
Controls	n=5	7.45	43	370	241	29	5	5	29	67

HI-6a	n=3	7.43	50	292	320	32	8	8	32	76
HI-6b	n=4	7.40	56	318	262	33	7	8	31	76
HI-6a / Atropine	n=4	7.40	53	330	296	32	7	7	31	74
HI-6b / Atropine	n=3	7.47	44	438	179	31	7	7	31	72
PYR / HI-6a	n=4	7.42	49	306	303	30	6	6	30	70
PYR / HI-6b	n=4	7.43	48	362	246	30	6	6	30	70
PYR / HI-6a / Atropine	n=5	7.40	56	375	223	32	7	7	31	75
PYR / HI-6b / Atropine	n=6	7.41	51	390	212	30	6	6	30	71
PYR	n=4	7.41	51	282	328	31	6	6	30	73

Table 5-Arterial blood gases in the HI-6 groups, 5 h post HI-6

		pH	pCO_2 (mmHg)	pO_2 (mmHg)	AaDpO2 (mmHg)	HCO^{3-} (mmol/L)	ABE (mmol/L)	SBE (mmol/L)	SBC (mmol/L)	tCO2 (Vol%)
Controls	n=5	7.41	48	382	226	29	5	5	29	68
HI-6a	n=3	7.44	51	318	294	33	8**	9	32**	78
HI-6b	n=4	7.42	53	321	223	33	8**	9	32**	78
HI-6a / Atropine	n=4	7.43	52	307	304	33	8	8	32	77
HI-6b / Atropine	n=3	7.46	45	428	199	31	7*	7	31**	72
PYR / HI-6a	n=4	7.40	53	302	303	31	6	7	30	73
PYR / HI-6b	n=4	7.42	50	311	296	31	7	7	31	74
PYR / HI-6a / Atropine	n=5	7.44	50	437	168	32	8**	8	31**	74
PYR / HI-6b / Atropine	n=6	7.40	54	325	265	32	6*	7	30*	74
PYR	n=4	7.39	55	347	259	31	6	7	30	74

Tables 1-5

Groups significantly different from controls:

* $p<0.05$

** $p<0.01$

Results

Hematology and Biochemistry

Table 6-Biochemistry, Electrolytes, Hematology: awake values in non-PYR groups compared to controls

	Mean Controls	Mean HI-6a	Mean HI-6b	Mean HI6-a / Atropine	Mean HI6-b / Atropine
	awake	awake	awake	awake	awake
TP g/l	58	61	61	61	58
ALB u/l	29	29	26	27	25
GLOB g/l	28	32	35	34	33
Ca++ mmol/l	2.58	2.67	2.35	2.37	2.40
Mg++ mmol/l	0.97	0.92	0.76	0.86	0.95
PHOS mmol/l	3.08	2.87	2.78	2.88	2.97
AST u/l	39	51	45	35	38
GGT u/l	50	42	40	37	49
ALKP u/l	283	263	261	215	273
TBIL mol/l	3	1	1	1	1
LDH u/l	2140	1769	1818	1711	1654
CK u/l	498	601	315	372	335
CHOL mmol/l	2.90	2.27	2.59	2.97	2.67
TRIG mmol/l	0.47	0.53	0.61	0.52	0.50
GLU mmol/l	6.20	6.01	6.74	6.16	5.97
AMYL u/l	444	545	500	571	715
LIPA u/l	11	3	10	7	12
CREA u/l	99	93	99	98	98
Urea/BUN mmol/l	3.73	4.29	4.46	4.06	5.53
NH3+ mol/l	104	96	77	84	161
URIC mol/l	6	6	6	6	8
Na+ mmol/l	147	145	143	142	145
K+ mmol/l	5.85	5.31	5.17	5.36	6.11
Cl^- mmol/l	107	104	105	104	104
Hct %	36.9	37.4	33.7	34.9	33.0
HGB g/dl	12.4	12.6	11.8	12.2	11.5
MCHC g/dl	33.5	33.8	34.9	34.9	34.9
WBC 10^9/L	14.7	16.0	16.2	17.7	22.9
GRAN 10^9/L	7.4	9.1	7.7	9.1	14.6
% GRAN	50	58	38	52	62
NEUT 10^9/L	4.8	7.7	6.2	7.4	16.7
EOS 10^9/L	1.0	1.4	1.7	1.7	1.8
L/M 10^9/L	7.3	6.9	8.5	8.6	8.3
% L/M	50	42	52	49	38
PLT 10^9/L	487	461	439	406	451
RETICS %	0.9	0.6	0.7	0.6	0.4

Table 7-Biochemistry, Electrolytes, Hematology: awake values in PYR groups compared to controls

	Mean Controls	Mean PYR / HI-6a	Mean PYR / HI-6b	Mean PYR / HI-6a / Atropine	Mean PYR / HI-6b / Atropine	Mean PYR

	awake	awake	awake	awake	awake	awake
TP g/l	58	57	60	61	59	59
ALB u/l	29	27	27	27	28	29
GLOB g/l	28	30	33	34	31	30
Ca++ mmol/l	2.58	2.39	2.37	2.41	2.44	2.55
Mg++ mmol/l	0.97	0.96	0.99	0.96	0.94	1.03
PHOS mmol/l	3.08	2.93	2.75	2.72	2.98	3.08
AST u/l	39	33	42	46	43	43
GGT u/l	50	60	50	44	48	57
ALKP u/l	283	300	329	267	270	277
TBIL mol/l	3	0	1	0	0	0
LDH u/l	2140	2032	2074	2087	1743	2293
CK u/l	498	290	328	442	562	414
CHOL mmol/l	2.90	2.45	2.43	2.33	2.56	2.56
TRIG mmol/l	0.47	0.52	0.57	0.44	0.59	0.59
GLU mmol/l	6.20	6.46	6.28	6.66	6.42	5.75
AMYL u/l	444	584	699	559	580	650
LIPA u/l	11	22	16	7	36	5
CREA u/l	99	106	108	103	103	108
Urea/BUN mmol/l	3.73	4.94	5.95	4.48	4.84	5.11
NH3+ mol/l	104	102	121	117	84	94
URIC umol/l	6	6	6	6	6	6
Na+ mmol/l	147	146	145	144	145	149
K+ mmol/l	5.85	6.00	6.02	5.42	6.36	6.55
Cl^- mmol/l	107	107	104	104	107	106
Hct %	36.9	33.1	42.2	35.2	34.5	36.5
HGB g/dl	12.4	11.7	13.7	12.1	11.8	12.5
MCHC g/dl	33.5	35.4	32.5	34.4	34.3	34.2
WBC 10^9/L	14.7	14.9	18.7	15.2	15.7	12.3
GRAN 10^9/L	7.4	7.7	12.4	6.9	7.5	6.6
% GRAN	50	52	66	45	47	56
NEUT 10^9/L	4.8	8.0	11.5	5.8	5.4	5.1
EOS 10^9/L	1.0	0.9	0.9	0.8	1.1	3.0
L/M 10^9/L	7.3	7.2	6.3	8.3	8.3	4.9
% L/M	50	48	34	55	53	31
PLT 10^9/L	487	449	507	459	433	475
RETICS %	0.9	0.3		0.5	0.6	0.5

Table 8-Biochemistry, Electrolytes, Hematology: values in non-PYR groups 5 hours after injection of HI-6 compared to controls

	Mean Controls	Mean HI-6a	Mean HI-6b	Mean HI-6a / Atropine	Mean HI-6b / Atropine
	5 h	5 h	5 h	5 h	5 h

TP g/l	46	45	49	51	43
ALB u/l	22	20	19	20	16
GLOB g/l	24	25	30	31	27
Ca++ mmol/l	2.36	2.01*	2.22	2.16	2.09*
Mg++ mmol/l	0.75	0.73	0.71	0.70	0.77
PHOS mmol/l	2.80	2.79	2.90	2.84	2.60
AST u/l	32	32	40	30	34
GGT u/l	36	32	29	28	33
ALKP u/l	211	206	189	170	203
TBIL mol/l	1	0	0	0	0
LDH u/l	1747	1368	1744	1560	1503
CK u/l	535	409	341	417	601
CHOL mmol/l	1.85	1.50	1.93	2.12	1.79
TRIG mmol/l	0.08	0.03	0.17*	0.13	0.11
GLU mmol/l	7.31	6.24	7.15	7.76	7.27
AMYL u/l	342	274	378	447	532
LIPA u/l	2	0	0	0	1
CREA u/l	89	87	95	91	93
Urea/BUN mmol/l	3.40	3.85	4.49	3.68	4.59
NH3+ mol/l	33	52	56	38	46
URIC mol/l	6	6	6	6	6
Na+ mmol/l	142	143	142	140	139
K+ mmol/l	4.72	4.08	4.69	4.32	4.75
Cl^- mmol/l	106	106	104	104	103
Hct %	27.1	22.5	26.1	27.0	24.0
HGB g/dl	9.6	7.4	9.1	9.6	8.3
MCHC g/dl	35.4	33.9	34.9	35.4	34.7
WBC 10^9/L	14.4	16.8	18.5	16.0	17.1
GRAN 10^9/L	12.1	14.5	15.1	13.0	14.3
% GRAN	83	86	81	81	83
NEUT 10^9/L	11.5	13.4	14.0	11.9	13.3
EOS 10^9/L	0.9	1.1	1.1	1.1	1.0
L/M 10^9/L	2.3	2.3	3.4	3.0	2.8
% L/M	17	14	19	20	17
PLT 10^9/L	247	260	243	215	282
RETICS %	0.9	0.5	0.6	0.3	

Grey values are values outside normal range. [Outside 95% CI for awake values]
Groups significantly different from controls:
* $p<0.05$
** $p<0.01$

Table 9-Biochemistry, Electrolytes, Hematology: values in PYR groups 5 hours after injection of HI-6 compared to controls

	Mean Controls	Mean PYR / HI-6a	Mean PYR / HI-6b	Mean PYR / HI-6a / Atropine	Mean PYR / HI-6b /	Mean PYR

					Atropine	
	5 h	5 h	5 h	5 h	5 h	5 h
TP g/l	46	46	48	48	45	46
ALB u/l	22	19	21	17	17	21
GLOB g/l	24	25	28	27	25	25
Ca++ mmol/l	2.36	2.13*	2.12*	2.12*	2.20	2.19
Mg++ mmol/l	0.75	0.79	0.80	0.82	0.80	0.84
PHOS mmol/l	2.80	2.67	2.45	2.61	2.78	2.74
AST u/l	32	41	34	34	39	36
GGT u/l	36	45	37	35	34	42
ALKP u/l	211	240	273	239	222	233
TBIL umol/l	1	0	0	0	0	0
LDH u/l	1747	2141	1937	1782	1660	2203
CK u/l	535	898	575	493	714	451
CHOL mmol/l	1.85	1.84	1.85	1.71	1.88	1.93
TRIG mmol/l	0.08	0.13	0.15*	0.09	0.14	0.23**
GLU mmol/l	7.31	7.69	6.95	8.71	8.79	6.99
AMYL u/l	342	443	531	433	432	496
LIPA u/l	2	6	4	10	8	0
CREA u/l	89	93	99	96	99	104
Urea/BUN mmol/l	3.40	4.40	4.97	4.19	4.36	4.22
NH3+ mol/l	33	40	45	38	48	27
URIC mol/l	6	6	6	6	6	6
Na+ mmol/l	142	141	140	141	141	144
K+ mmol/l	4.72	4.59	4.52	4.84	4.67	4.73
Cl^- mmol/l	106	106	105	101	103	106
Hct %	27.1	24.6	28.9	27.5	26.1	26.5
HGB g/dl	9.6	8.6	9.9	9.4	8.9	8.9
MCHC g/dl	35.4	34.7	34.1	34.4	34.2	33.5
WBC 10^9/L	14.4	15.9	14.4	12.8	14.7	11.8
GRAN 10^9/L	12.1	13.0	11.7	10.0	11.5	9.3
% GRAN	83	80	81	76	75	78
NEUT 10^9/L	11.5	11.0		12.7	13.7	8.3
EOS 10^9/L	0.9	0.7		1.2	0.7	1.2
L/M 10^9/L	2.3	2.9	2.8	2.8	3.1	2.6
% L/M	17	21	19	24	25	22
PLT 10^9/L	247	286	268	284	265	290
RETICS %	0.9	0.4	0.3	0.4	0.5	0.5

Grey values are values outside normal range. [Outside 95% CI for awake values]
Groups significantly different from controls:
* $p<0.05$
** $p<0.01$

Results

Physiology

Figure 7-HI-6a / HI-6b compared to controls: mean arterial pressure (MAP)

Figure 8-HI6-a / HI6-b compared to controls: Heart Rate (HR)

Figure 9-HI-6a / HI6-b compared to controls: Respiration Rate (f)

Figure 10-HI-6a / HI-6b compared to controls: $ETCO_2$

Figure 11-HI-6a / HI-6b compared to controls: SO_2

Figure 12-HI-6a / HI-6b compared to controls: Temperature

Figure 13-HI-6a / HI-6b with atropine compared to controls: MAP

Figure 14-HI-6a / HI-6b with atropine compared to controls: Heart Rate

Figure 15 HI-6a / HI-6b with atropine compared to controls: Respiration Rate

Figure 16-HI-6a / HI-6b with atropine compared to controls: $ETCO_2$

Figure 17-HI-6a / HI-6b with atropine compared to controls: SO_2

Figure 18-HI-6a / HI-6b with atropine compared to controls: Temperature

Figure 19-PYR followed by HI-6a / HI-6b compared to controls and PYR only: MAP

Figure 20-PYR followed by HI-6a / HI-6b compared to controls and PYR only: Heart Rate

Figure 21-PYR followed by HI-6a / HI-6b compared to controls and PYR only: Respiration Rate

Figure 22-PYR followed by HI-6a / HI-6b compared to controls and PYR only: $ETCO_2$

Figure 23-PYR followed by HI-6a / HI-6b compared to controls and PYR only: SO_2

Figure 24-PYR followed by HI-6a / HI-6b compared to controls and PYR only: Temperature

Figure 25-PYR followed by HI-6a / HI-6b with atropine compared to controls and PYR only: MAP

Figure 26-PYR followed by HI-6a / HI-6b with atropine compared to controls and PYR only: Heart Rate

Figure 27-PYR followed by HI-6a / HI-6b with atropine compared to controls and PYR only: Respiration Rate

Figure 28-PYR followed by HI-6a / HI-6b with atropine compared to controls and PYR only: $ETCO_2$

Figure 29-PYR followed by HI-6a / HI-6b with atropine compared to controls and PYR only: SO_2

Figure 30-PYR followed by HI-6a / HI-6b with atropine compared to controls and PYR only: Temperature

Figure 31-HI-6a / HI-6b compared to controls: VT_I

Figure 32-HI-6a / HI-6b compared to Controls: VT_E

Figure 33-HI-6a / HI-6b compared to controls: f

Figure 34-HI-6a / HI-6b compared to controls: VE

Figure 35-HI-6a / HI-6b compared to controls: PIFR

Figure 36-HI-6a / HI-6b compared to Controls: PEFR

Figure 37-HI-6a / HI-6b compared to controls: RAW_E

Figure 38-HI-6a / HI-6b compared to controls: RAW_M

Figure 39-HI-6a / HI-6b compared to controls: WOB

Figure 40-HI-6a / HI-6b compared to controls: C_{DYN}

Figure 41-HI-6a / HI-6b compared to controls: P0.1

Figure 42-HI-6a / HI-6b compared to controls: T_I T_{TOT}

Figure 43-HI-6a / HI-6b with atropine compared to controls: VT_I

Figure 44-HI-6a / HI-6b with atropine compared to controls: VT_{IE}

Figure 45-HI-6a / HI-6b with atropine compared to controls: f

Figure 46-HI-6a / HI-6b with atropine compared to controls: VE

Figure 47-HI-6a / HI-6b with atropine compared to controls: PIFR

Figure 48-HI-6a / HI-6b with atropine compared to controls: PEFR

Figure 49-HI-6a / HI-6b with atropine compared to controls: RAW_E

Figure 50-HI-6a / HI-6b with atropine compared to controls: RAW_M

Figure 51-HI-6a / HI-6b with atropine compared to controls: WOB

Figure 52-HI-6a / HI-6b with atropine compared to controls: C_{DYN}

Figure 53-HI-6a / HI-6b with atropine compared to controls: P0.1

Figure 54-HI-6a / HI-6b with atropine compared to controls: $T_I T_{TOT}$

Figure 55-PYR followed by HI-6a / HI-6b compared to controls and PYR only: VT_I

Figure 56-PYR followed by HI-6a / HI-6b compared to controls and PYR only: VT_E

Figure 57-PYR followed by HI-6a / HI-6b compared to controls and PYR only: f

Figure 58-PYR followed by HI-6a / HI-6b compared to controls and PYR only: VE

Figure 59-PYR followed by HI-6a / HI-6b compared to controls and PYR only: PIFR

Figure 60-PYR followed by HI-6a / HI-6b compared to controls and PYR only: PEFR

Figure 61-PYR followed by HI-6a / HI-6b compared to controls and PYR only: RAW_E

Figure 62-PYR followed by HI-6a / HI-6b compared to controls and PYR only: RAW_M

Figure 63-PYR followed by HI-6a / HI-6b compared to controls and PYR only: WOB

Figure 64-PYR followed by HI-6a / HI-6b compared to controls and PYR only: C_{DYN}

Figure 65-PYR followed by HI-6a / HI-6b compared to controls and PYR only: P0.1

Figure 66-PYR followed by HI-6a / HI-6b compared to controls and PYR only: $T_I T_{TOT}$

Figure 67-PYR followed by HI-6a / HI-6b with atropine compared to controls and PYR only: VT_I

Figure 68-PYR followed by HI-6a / HI-6b with atropine compared to controls and PYR only: VT_E

Figure 69-PYR followed by HI-6a / HI-6b with atropine compared to controls and PYR only: f

Figure 70-PYR followed by HI-6a / HI-6b with atropine compared to controls and PYR only: VE

Figure 71-PYR followed by HI-6a / HI-6b with atropine compared to controls and PYR only: PIFR

Figure 72-PYR followed by HI-6a / HI-6b with atropine compared to controls and PYR only: PEFR

Figure 73-PYR followed by HI-6a / HI-6b with atropine compared to controls and PYR only: RAW_E

Figure 74-PYR followed by HI-6a / HI-6b with atropine compared to controls and PYR only: RAW_M

Figure 75-PYR followed by HI-6a / HI-6b with atropine compared to controls and PYR only: WOB

Figure 76-PYR followed by HI-6a / HI-6b with atropine compared to controls and PYR only: C_{DYN}

Figure 77-PYR followed by HI-6a / HI-6b with atropine compared to controls and PYR only: P0.1

Figure 78-PYR followed by HI-6a / HI-6b with atropine compared to controls and PYR only: $T_I T_{TOT}$

Discussion

Cholinesterase (ChE) activity

Induction of anesthesia results in a ~15% inhibition of ChE activity [Figures 1-6]. This may be explained by splenic contracture associated with stress and followed by relaxation post-induction [1].

Intramuscular injection of either salt of HI-6 "reverses" the ChE inhibition although this "reversal" effect lies within ~1 SD (above) of control values [Figure 2]. This may be due muscarinic receptor stimulation [2]. A combination of atropine plus either salt lessens the "reversal" somewhat. Values for both HI-6a and HI-6b still lie within 1 SD of each other and within ~ 1SD of control values [Figure 3].

PYR has direct cholinergic agonist effects in addition to inhibition of ChE. 1 mg/kg of iv Pyridostigmine (PYR) results in a further ~30% inhibition of ChE activity 1 hour post-injection [Figures 4-6]. This degree of inhibition is consistent with values found by other researchers utilizing anesthetized domestic swine [3] and the degree of inhibition sought when PYR is administered prophylactically to humans [4].

Injection of either salt of HI-6 +/- atropine "reverses" the ChE inhibitory effect of PYR to within ~ 1SD (lower) control values [Figures 4-6]. There do not appear to be significant differences between either the HI-6 salts or between groups which received atropine and those which did not [Figure 4].

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3. Modell HI. Influence of anticholinesterase on distribution of ventilation and gas exchange. Pharmacol Biochem Behaviour 1991; 40:17-20.
4. Keeler JR. Interactions between nerve agent pretreatment and drugs commonly used in combat anesthesia. Mil Med 1990; 155:527-33.

Discussion

Arterial Blood Gases (ABG)

Table 1 illustrates the range of ABG results in 42 animals at the same time point. Note is made of the relative consistency of pH, Pco2 and the variability in Po2, A-aDo2 and HCO3. There are no statistically significant differences between the experimental groups

Administration of im PYR alone and PYR/HI-6 does not result in values, which are statistically different from, controls [Tables 2-5]

At 1 hour [Table 3] the total CO2 is statistically elevated in both HI-6 groups. The difference in level of significance is probably related to the difference in group size. This value is also significantly different in the HI-6a/Atrpine group.

At 5 hours [Table 5] the arterial base excess [ABE] and standard bicarbonate [SBC] are significantly elevated in the HI-6, HI-6/Atropine and PYR/HI-6/Atropine groups. Differences in level of significance may reflect differences in group size.

Of interest is the observation that HI-6a seems to produce more statistically different values than does HI-6b.

1. Shapiro BA, Harrison RA, Walton JR: *Clinical Application of Blood Gases*, ed.3, Chicago, Year Book Medical Publishers, 1982.

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Discussion

Hematology and Biochemistry

There are no differences between groups when awake values are examined [Tables 6-7] Awake values are similar to those published elsewhere [1]. Administration of HI-6 causes a relative hypertriglyceridemia, which is of questionable clinical significance. Administration of HI-6a causes a relative hypocalcemia as does administration of HI-6b/atropine [Table 8]. Administration of PYR results in a statistically significant hypertriglyceridemia when administered alone or with HI-6b [Table 9], which is of questionable clinical

significance. Administration of PYR in conjunction with either HI-6 salt and with HI-6a/atropine also causes hypocalcemia [Table 9].

IDEXX equipment allows testing for a battery of biochemical and hematological parameters. Although specifically designed for veterinary use this equipment allows testing which is analogous to that commonly used for humans.

Calcium and phosphate are the two minerals, which are of diagnostic importance in all species while magnesium is usually only important in ruminants.

Normal plasma calcium concentration is 2-3 mmol/l. It is of major importance in transmission at the neuromuscular junction and in the propagation of the contraction impulse within the muscle. The relevant differential diagnosis for a relative hypocalcemia includes:

1-expansion of the total vascular space with iv fluids

2-hypoalbuminemia

3-renal failure-usually chronic/associated with increased PHOS

4-acute pancreatitis-usually after 5-7 days.

5-anticoagulants-EDTA, oxalate, citrate

5-anticoagulants-EDTA, oxalate, citrate

In fat depots fat is stored as TRIG, which consists of 3 fatty acids esterfied to a glycerol unit. Normal fat mobilization is stimulated by adrenaline and involves lipases and esterases within the fat depot to split the fatty acids off. Free fatty acids and glycerol are released into the plasma while plasma TRIG remains unaffected. The relevant differential diagnosis for hypertriglyceridemia includes:

1-renal failure

2-acute necrotizing pancreatitis.

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2. Bush BM (1991) *Interpretation of Laboratory Results for Small animal Clinicians*, Blackwell Science, Oxford.

3. Duncan JR, Prasse KW, Mahaffey EA (1994) *Veterinary Laboratory Medicine Clinical pathology*, 3rd edn Iowa State University Press, Ames IA.

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Discussion

Physiology

Both HI-6 salts produce similar trends when the effects on physiological endpoints are compared to controls. There appears to be no significant clinical differences in the groups [Figures 7-12].

The addition of atropine to both HI-6 salts produces similar trends when the effects on physiological endpoints are compared to controls. There appears to be no significant clinical differences in the groups although the effects appear to be more variable with HI-6b [Figures 13-18].

Injection of PYR produces early changes in MAP [Figure 19] and elevates f to above control levels [Figure 21] with resultant decrease of ETCO2 to below control levels [Figure 22]. Of interest is the stability of both cardiac rate [Figure 20] and temperature [Figure 24]. The cardiorespiratory/temperature findings contrast with those found in conscious swine receiving physostigmine [1].

Both HI-6 salts produce similar trends when the effects on physiological endpoints are compared to controls. There appears to be no significant clinical differences in the groups.

Addition of atropine to both HI-6 salts results in an early increase in MAP without an increase in heart relative to controls [Figures 25-26]. This finding contrasts with the finding of raised MAP and heart rate when atropine is administered to conscious swine [2]. Both HI-6 salts/atropine produce similar trends when the effects on physiological endpoints are compared to controls. There appears to be no significant clinical differences in the groups.

Both HI-6 salts show similar effects on respiratory endpoints. HI-6b produces less deviation from control values, notably, VT, f, RAW, WOB, C_{DYN} and $P_{0.1}$ [Figures 31-42].

The addition of atropine to either HI-6 salt lessens the deviation from control values. A notable exception being the effect on RAW when HI-6a and atropine are combined [Figures 43-54]. HI-6b produces less deviation from control values.

As has been reported in conscious swine, PYR alone increased airway resistance [3]. This probably accounts for the increase in WOB and decrease in compliance. This may explain, at least partially, the

increase in central respiratory drive (CRD) reflected by the mouth occlusion pressures [Figures 61,62,63,64,65].

Although trends remain similar for both HI-6 salts, the addition of PYR to HI-6b results in slightly increased minute ventilation (V_E) secondary to increased V_T. The addition of PYR is associated with an increase in resistance (R), WOB and increased CRD ($P_{0.1s}$) [compare Figures 37 Vs 61,38 Vs 62,39 Vs 63,41 Vs 65]. Addition of atropine modifies the effects of PYR towards control values [Figures 67-78].

1. Stemler FW, Corcoran KD, Parrish JH et al. Effects of physostigmine on the cardiopulmonary system of conscious pigs. Fund Appl Toxicology 1990; 14:96-103.
2. Wade CE, Waring PP, Trail DS et al. Effects of atropine, 2-PAM, or pyridostigmine in euvolemic or hemorrhagic conscious swine. Mil Med 1988; 9:470-6.
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Conclusions/Suggestions

Cholinesterase (ChE) activity

There do not appear to be significant differences between the HI-6 salts +/- atropine when " reversal" of either anesthesia induced or pyridostigmine induced inhibition of ChE activity is examined.

PYR (1 mg/kg iv) results in inhibition of ChE levels to those used in humans for nerve agent pretreatment.

Suggestion: The effect of HI-6 on splenic contracture should be assessed using serial hematocrit [HCT] taken over the time course of the "reversal" process.

Suggestion: The efficacy of HI-6 in reversing non-depolarizing muscle relaxants should be assessed and compared to conventional reversal agents like edrophonium, neostigmine, etc.,

Conclusions/Suggestions

Arterial Blood Gases (ABG)

Both HI-6 salts produce similar changes in ABG results. These are of questionable clinical significance.

Conclusions/Suggestions

Hematology and Biochemistry

Administration of either HI-6 salt produces minimal impact on clinical biochemistry with no clear difference between the two salts. Hypocalcemia could become clinically significant acutely whereas the hypertriglyceridemia probably would not.

Suggestion: Administration of 2-PAM to euvolemic conscious swine produces a transient rise in blood lactate levels. Such an increase in lactate during hemorrhage might be detrimental if adequate buffering capabilities are not available. Consideration should be given to assessment of the effect of both salts of HI-6 on blood lactate levels in both euvolemic and hypovolemic animals.

Conclusions/Suggestions

Physiology

Suggestion: Consideration should be given to comparing the physiologic effects of physostigmine to those of pyridostigmine at doses which produce equal ChE activity inhibition in the anesthetized DSM. A suggested starting dose for iv pyridostigmine salycilate is 5 g/kg/min over 2 h.

Suggestion: Addition of PYR to HI-6b results in potentially deleterious effects on airway resistance. This should be investigated further

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A well-developed anesthetized domestic swine model has been utilized to study the following effect of chemical weapons (CW):

01- Comparative bioavailability of different HI-6 salt forms in the domestic pig
The bioavailability of the dihydrochloride salt of HI-6a (currently utilized) was compared to that of the dimethanesulfonate salt (HI-6b). The latter is more water-soluble at low temperatures. The salts appear similarly clinically, biochemically or hematologically in the "equivalent" doses administered (HI-6 dihychloride = 500mg, HI-6 dimethanesulfonate = 633mg.) A notable exception might be the observation that the combination of pyridostigmine (PYR)/HI-6b increases airway resistance. Timed plasma and urine samples were collected for HPLC analysis.

Nous avons utilisé un modèle bien établi de porc domestique anesthésié pour étudier les effets ci dessous d'armes chimiques (CW) :

01- Biodisponibilité comparative de différentes formes salines de HI-6 présentes dans un porc domestique.
Nous avons comparé la biodisponibilité du sel de dichlorhydrate de HI-6a (actuellement utilisé) à celle du sel de diméthanesulfonate (HI-6b), qui est plus hydrosoluble à une basse température. Les sels semblent similaires sur le plan clinique, biochimique ou hématologique d'après les doses « équivalentes » administrées (dichlorhydrate de HI-6a = 500 mg et diméthanesulfonate HI-6b = 633 mg). La seule exception notable pourrait être l'observation selon laquelle la combinaison de pyridostigmine et de HI-6b augmente la résistance des voies aériennes. Des échantillons de plasma et d'urine ont été recueillis par intervalles aux fins d'analyse par CLHP.

14. KEYWORDS, DESCRIPTORS or IDENTIFIERS (Technically meaningful terms or short phrases that characterize a document and could be helpful in cataloguing the document. They should be selected so that no security classification is required. Identifiers, such as equipment model designation, trade name, military project code name, geographic location may also be included. If possible keywords should be selected from a published thesaurus, e.g. Thesaurus of Engineering and Scientific Terms (TEST) and that thesaurus identified. If it is not possible to select indexing terms which are Unclassified, the classification of each should be indicated as with the title.)

chemical weapons