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PRECLINICAL TOXICOLOGY OF NEW DRUGS

ANNUAL AND FINAL REPORT

WILLIAM M. KLUWE

July 31, 1988

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
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The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents.

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TABLE OF CONTENTS

<u>Subject</u>	<u>Page</u>
SUMMARY.	1
FOREWORD	1
TABLE OF CONTENTS.	2
PROBLEM, BACKGROUND AND APPROACH	3
RESULTS AND DISCUSSION	4
CONCLUSIONS.	16
DISTRIBUTION LIST.	17

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SUMMARY

A number of potential therapeutic drugs of interest to the U.S. Army Medical Research and Development Command (USAMRDC) were evaluated in animal studies to predict potential toxicity in humans. The principal materials studied were WR238605, Succinate, an 8-aminoquinoline derivative being developed as a possible antimalarial drug, and pyridostigmine, a reversible acetylcholinesterase inhibitor. The studies performed included standard acute, subchronic and chronic designs, as well as unique studies with pyridostigmine to evaluate subtle morphological and functional effects on the neuromuscular junction.

The studies were conducted by board-certified professional scientists (toxicologists, veterinarians, pathologists) in facilities accredited by the American Association for the Accreditation of Laboratory Animal Care (AAALAC). All studies were conducted in accordance with the U.S. Food and Drug Administration's Good Laboratory Practices guidelines (GLP) (21 CFR Par. 58), and protocols, critical events and reports were reviewed by Battelle's Quality Assurance Unit.

FOREWORD

Citations of commercial organizations or trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals", prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHHS Publication No. (NIH) 85-23, Revised 1985).

PROBLEM, BACKGROUND, AND APPROACH

The U.S. Army Medical Research and Development Command (USAMRDC) is developing therapeutic agents for use in protecting or improving the health of military personnel. In order to protect the U.S. citizenry from undue risk, the U.S. Food and Drug Administration (FDA) has been charged with regulatory responsibility for approval of drugs for human or veterinary use. Although USAMRDC is not bound to strict compliance with FDA regulations, it is their intent to use the FDA regulations as guidelines for preclinical development of potential therapeutics.

The FDA takes a cautious approach to the safety evaluation of potential new drugs, requiring a variety of preclinical toxicology tests to be performed in order to estimate toxic response in humans. Since no single model species or test is uniquely predictive of human response, many different types of toxicology studies may be required before FDA approval. The types and numbers of required studies are partially dependent on the anticipated length and doses to be used in humans, and similarity to agents of known toxic potential.

Upon completion of the preclinical toxicology studies, the USAMRDC will file a Notice of Clinical Exemption for an Investigational New Drug (IND) with the FDA. The IND application will reference all preclinical toxicology work, as well as relevant pharmacology, chemistry and other information. Should the FDA decide that the potential benefits of the proposed new drug justify the risks suggested by the preclinical toxicology studies, limited clinical trials will be approved. More focused and longer-term animal studies may also be required at this time to predict the potential for chronic toxicity or irreversible effects in humans. The human clinical trial data and the longer-term (or other) animals studies will then be used to support a New Drug Application (NDA) to the FDA. If the NDA is approved, then the therapeutic agent can be introduced into general clinical use.

Because of the importance of the preclinical toxicology studies with regard to veracity of performance and reporting; and the legal responsibility of the performing (Battelle), sponsoring (USAMRDC), and reviewing

(FDA) organizations to present the data in a forthright manner, it is essential that these studies be performed by a reputable organization with a competent, experienced staff and adequate facilities. In recognition of the need to document proper performance and data recording, the FDA issued in 1978 formal Good Laboratory Practices (GLP) regulations which must be adhered to when studies are performed for submission of preclinical toxicology studies to the FDA. The studies described in this Final Contract Report have all been conducted in accord with GLPs.

RESULTS AND DISCUSSION

Several studies were completed under this contract. A list of these studies is contained in Table 1, and each is described briefly in the following pages.

TABLE 1. STUDIES COMPLETED UNDER THIS CONTRACT

Study Number	Title/Description
G-8740-1000	Method Development and Intramuscular Irritation Study in Rabbits with Sodium Thiosulfate/Sodium Nitrite
G-8740-1100	Pyrogen Study with Liposomal Drug Delivery System
G-8740-1200	Ames Bacterial Mutagenicity Test of WR238605,Succinate
G-8740-1300	WR238605,Succinate, Rat, Oral, LD50
G-8740-1400	WR238605,Succinate, Rat, IP, LD50
G-8740-1500	WR238605,Succinate, Mouse, Oral, LD50
G-8740-1600	WR238605,Succinate, Mouse, IP, LD50
G-8740-1700	WR238605,Succinate, Rabbit, Oral, LD50
G-8740-1800	WR238605,Succinate, Rabbit, IP, LD50

TABLE 1. STUDIES COMPLETED UNDER THIS CONTRACT (Continued)

Study Number	Title/Description
G-8740-1900	Primaquine, Mouse, Oral, LD50
G-8740-2000	WR238605, Succinate, Guinea Pig, Oral, LD 50
G-8740-2100	WR238605, Succinate, Guinea Pig, IP, LD50
G-8740-2200	WR238605, Succinate, Dog, Approximate Lethal Dose
G-8740-2300	Twenty-eight Day Toxicity Study of WR238605, Succinate in Dogs
G-8740-2400	Fourteen Day Rat Pilot Study with WR238605, Succinate
G-8740-2500	WR238605, Succinate, Rat, 28 Day
G-8740-2600	Effects of Single Intravenous and Oral Doses of Pyridostigmine Bromide on Neuromuscular Junction Morphology in Sprague-Dawley Rats
G-8740-2700	Effects of Single Oral Doses of Pyridostigmine Bromide and Muscle Activity on Neuromuscular Junction Morphology in Male, Sprague-Dawley Rats
G-8740-2800	14-Day Pilot Oral Toxicity Study of Pyridostigmine in the Rat
G-8740-3200	A 14-Day Pilot Dose Range Oral Toxicity Study in Dogs with Pyridostigmine
G-8740-3300	<u>In Vitro</u> and <u>In Vivo</u> Effects of Pyridostigmine Bromide on Dog Red Blood Cell Acetylcholinesterase Activity
G-8740-3400	Pilot Repeated Oral Dose Toxicity Study in Dogs to Examine Pyridostigmine Inhibition of Red Blood Cell Acetylcholinesterase
G-8740-3500	Acute Oral Toxicity Study of WR238605, Succinate in Fischer 344 Rats
G-8740-3600	90-Day Oral Dose Toxicity Study of Pyridostigmine in Dogs

*Study numbers 2900, 3000 and 3100 not used.

G-8740-1000 Sodium Thiosulfate/Sodium Nitrite:
Method Development and Intramuscular
Irritation Study in Rabbits

Sodium thiosulfate and sodium nitrite were scheduled for study [intramuscular (IM) injection] to determine the potential of these chemicals to cause subsequent muscle irritation. Therefore, an animal model was developed in rabbits to study the potential for therapeutic compounds to cause muscle irritation following IM injection. Aqueous solutions of sodium thiosulfate, sodium nitrite, or the vehicle alone (sterile water) were injected into the Vastus lateralis muscle of New Zealand Albino rabbits. Muscle irritation was assessed by both gross and microscopic evaluation, and repair capacity was documented. Minimal inflammation and tissue necrosis were observed at the injection site following the administration of either sodium nitrite or sterile water (vehicle), and muscle healing (repair) was evident. Sodium thiosulfate produced more severe muscular lesions, and the capacity for tissue repair was exceeded with the larger doses.

G-8740-1100 Liposomal Drug Delivery
System: Pyrogen Study

A USP Rabbit Pyrogen Test was performed on a suspension of sample liposomes, a novel method of drug delivery of potential use in the administration of antimalarial agents. The USP Pyrogen test is most commonly used to test commercial drug formulations for the presence of pyrogenic (fever-inducing) bacterial products. It involves intravenous administration of the test article in rabbits and body (rectal) temperature monitoring. In addition to the pyrogen test, the drug delivery system-- liposomes in hypertonic saline--was characterized as to pH, osmolarity, and liposome size (median size and size distribution patterns).

In brief, the intravenous administration of the liposomes to eight New Zealand Albino rabbits produced elevations (greater than 0.6°C) of rectal temperatures in three of the animals. The cumulative total increase in rectal temperatures was 4.1°C, exceeding the USP limit of 3.7°C, and the liposomes are therefore classified as pyrogens under USP standards. These

data suggest the possibility of bacterial contamination of the liposomes during their manufacture.

G-8740-1200 Ames Bacterial Mutagenicity
Test of WR238605, Succinate

The test article, WR238605, Succinate, was tested for mutagenic activity in a standard bacterial assay system--both with and without metabolic activation--at Battelle's Pacific Northwest Laboratories. More specifically, the abilities of WR238605, Succinate at additions of 0.1 $\mu\text{g}/\text{plate}$ to 1000 $\mu\text{g}/\text{plate}$ to produce histidine revertant colonies of Salmonella tester strains, TA 98, TA 100, TA 1537, or TA 1538 were evaluated in standard plate incorporation assays. Cytotoxicity was observed at the higher concentrations. No mutagenic activity was observed at lower, non-toxic concentrations.

G-8740-1300 Acute (LD50) Oral Toxicity Study
of WR238605, Succinate in Rats

Pilot and definitive acute oral toxicity studies of WR238605, Succinate suspended in a vehicle of 1% (w/v) methylcellulose/0.4% (v/v) Tween 80 were conducted in Fischer 344 rats. The dose ranges encompassed 66 mg/kg to 1400 mg/kg.

G-8740-1400 Acute (LD50) Intraperitoneal Toxicity
Study of WR238605, Succinate in Rats

Pilot and definitive acute intraperitoneal toxicity studies of WR238605, Succinate suspended in a vehicle of 1% (w/v) methylcellulose/0.4% (v/v) Tween 80 were conducted in Fischer 344 rats. The dose ranges encompassed 0 mg/kg (vehicle control) to 320 mg/kg.

G-8740-1500 Acute (LD50) Oral Toxicity Study of WR238605, Succinate in Mice

Pilot and definitive acute oral toxicity studies of WR238605, Succinate suspended in a vehicle of 1% (w/v) methylcellulose/0.4% (v/v) Tween 80 were conducted in B6C3F1 mice. The dose range encompassed 62.5 mg/kg to 1000 mg/kg. Additional groups of mice had to be added to the original study design.

G-8740-1600 Acute (LD50) Intraperitoneal Toxicity Study of WR238605, Succinate in Mice

Pilot and definitive acute intraperitoneal toxicity studies of WR238605, Succinate suspended in a vehicle of 1% (w/v) methylcellulose/0.4% (v/v) Tween 80 were conducted in B6C3F1 mice. The dose ranges encompassed 10 mg/kg to 160 mg/kg. Additional groups of mice had to be added to the original study design.

G-8740-1700 Acute (LD50) Oral Toxicity Study of WR238605, Succinate in Rabbits

Pilot and definitive acute oral toxicity studies of WR238605, Succinate suspended in a vehicle of 1% (w/v) methylcellulose/0.4% (v/v) Tween 80 were conducted in New Zealand Albino rabbits. The dose range encompassed 50 mg/kg to 1107 mg/kg. Additional groups of rabbits had to be added to the original study design.

G-8740-1800 Acute (LD50) Intraperitoneal Toxicity Study of WR238605, Succinate in Rabbits

Pilot and definitive acute intraperitoneal toxicity studies of WR238605, Succinate suspended in a vehicle of 1% (w/v) methylcellulose/0.4% (v/v) Tween 80 were conducted in New Zealand Albino rabbits. The dose range encompassed 5 mg/kg to 80 mg/kg. Additional groups of rabbits had to be added to the original study design.

G-8740-1900 Acute (LD50) Oral Toxicity Study of Primaquine in Mice

An acute, 14 day LD50 study of primaquine administered orally (by gavage) as a solution in sterile water was conducted in B6C3F1 mice. The dose ranges encompassed 114 mg/kg to 198 mg/kg. Primaquine was found to produce extensive swelling and protrusion of the tongue, to the extent that it may have contributed to death by precluding ingestion of food or water.

G-8740-2000 Acute (LD50) Oral Toxicity Study of WR238605, Succinate in Guinea Pigs

Pilot and definitive acute oral toxicity studies of WR238605, Succinate suspended in a vehicle of 1% (w/v) methylcellulose/0.4% (v/v) Tween 80 were conducted in Hartley Albino guinea pigs. The dose ranges encompassed 200 mg/kg to 800 mg/kg. Additional groups of guinea pigs had to be added to the original study design.

G-8740-2100 Acute (LD50) Intraperitoneal Toxicity Study of WR238605, Succinate in Guinea Pigs

Pilot and definitive acute intraperitoneal toxicity studies of WR238605, Succinate suspended in a vehicle of 1% (w/v) methylcellulose/0.4% (v/v) Tween 80 were conducted in Hartley Albino guinea pigs. The dose ranges encompassed 10 mg/kg to 100 mg/kg. Additional groups of guinea pigs had to be added to the original study design.

Vehicle for WR238605, Succinate

Personnel at W.R.A.I.R. had requested that a suspending vehicle of 0.2% (w/v) methylcellulose/0.4% (v/v) Tween 80 be used with structural analogs of WR238605, Succinate. This vehicle was found to produce insufficiently-stable suspensions. Several other vehicles were evaluated, and after discussion with the C.O.T.R., a vehicle of 1% (w/v) methylcellulose/0.4% (v/v) Tween 80 in sterile distilled water was selected for use.

WR238605, Succinate/Vehicle-Induced Peritonitis

Abdominal adhesions were detected in many of the rats, mice, rabbits, and guinea pigs in the intraperitoneal WR238605, Succinate studies, both in the vehicle control and in the WR238605, Succinate treated groups. After discussions with the C.O.T.R., the decision was made to microscopically evaluate the affected tissues. The microscopic analyses confirmed the presence of peritonitis, while special stains documented a lack of bacterial involvement with the lesions. Further studies indicated that the vehicle (1% methylcellulose/0.4% Tween 80 in sterile distilled water) caused chemical peritonitis. The C.O.T.R. thus directed Battelle to discontinue the intraperitoneal studies.

G-8740-2200 Approximate Lethal Dose Toxicity Study of WR238605, Succinate in Dogs

Beagle dogs received WR238605, Succinate orally (gelatin capsule) for up to 18 days at daily doses of 12.5 to 120 mg/kg. This study will be part of the basis for selecting doses for a 28 day study in dogs (G-8740-2300).

G-8740-2300 Twenty-eight Day Toxicity Study of WR238605, Succinate in Dogs

Groups of 3 male and 3 female beagle dogs received daily doses of 0 (vehicle), 3.9, 7.9 or 15.7 mg/kg WR238605, Succinate, orally (capsule) for 28 consecutive days. The major clinical sign of toxicity was bluish, cyanotic appearance of the mucous membranes in all WR238605 dose groups by the second week of treatment. This was correlated with a dose-dependent methemoglobinemia that achieved a maximum during the second or third week of treatment. Methemoglobin values as percentages of total hemoglobin during the final week of study were 0.0, 15.9, 20.9 and 31.0 percent for males in the control, 3.9, 7.9 and 15.7 mg/kg dose groups, respectively, and 0.1, 16.6, 23.7 and 24.7 percent for females in the control, 3.9, 7.9 and

15.7 mg/kg dose groups, respectively. Drug-related lesions observed upon microscopic examination of tissues from the dogs included mild inflammation of lung interstitium and alveolar surfaces, mild inflammation of the liver, and mild lymphoid depletion and lymphoid necrosis in several tissues.

These data indicate that WR238605, Succinate, is a fairly toxic drug with a strong potential for producing methemoglobinemia. Since substantial methemoglobinemia (approximately 16-17 percent of total hemoglobin) was apparent at the lowest dose evaluated, 3.9 mg/kg/day, a no-effect dose was not established in this study.

G-8740-2400 Fourteen Day Pilot Study of
WR238605, Succinate in Rats

The test compound, WR238605, Succinate was administered once daily for 14 consecutive days by gavage in 1% (w/v) methylcellulose/0.4% (v/v) Tween 80 vehicle to Fischer 344 rats at the estimated acute LD10 dose and fractions thereof (360, 180, 70, 45, 22.5 mg/kg). Deaths occurred in the 90, 180, and 360 mg/kg/day groups. The frequency of death was directly related to dose, while the time to death was inversely related to dose. Clinical symptoms of malaise (hunched posture, labored respiration, lethargy, ataxia, weight loss, or poor weight gain) were observed at all doses except 22.5 mg/kg/day; time to onset was inversely related to dose. Food consumption was decreased in a dose-related fashion. No visible abnormalities of the tissues were observed at necropsy.

These data indicate a strong potential for cumulative toxicity of WR238605, Succinate when administered daily. Whether or not this could be due to compound accumulation in target tissues is at present unknown. Because of the progressive debilitation observed even at the next to the lowest dose, 45 mg/kg/day, the recommendation was made that the high dose for the subsequent 28 day study be no lower than 22.5 and no higher than 30 mg/kg/day.

G-8740-2500 28 Day Oral Toxicity Study of WR238605, Succinate
in Fischer 344 Rats

Male and female rats received 0 (vehicle), 3.8, 11.5, 20.5, 34.6, or 38.5 mg/kg of WR238605 daily by gavage for 28 days. Some of the rats receiving 34.6 mg/kg were allowed a 14 day recovery period to assess reversibility of drug effects. Signs of intoxication during treatment included pallor, ruffled fur, abnormal posture, and dose-related decreases in food consumption and body weight. Dose-related increases in methemoglobin, circulating reticulocytes and nucleated red blood cells were observed, along with increased liver and spleen weights. Pulmonary edema, mild lymphoid depletion of the spleen, and aspermatogenesis were noted on microscopic examination of the tissues.

The hematologic effects occurred in a dose-related fashion and were observed even at the lowest dose, 3.8 mg/kg. Thus, a no-effect dose was not established. In addition, the microscopic lesions (e.g., splenic lymphoid depletion) were still apparent in the recovery group animals, indicating less than complete reversal of toxicity within the 14 day recovery period.

G-8740-2600 Extent and Reversibility of Acute Effects of
G-8740-2700 Pyridostigmine, Including Neuromuscular
Junction Lesions

Sprague-Dawley rats received fractions of the LD50 dose of pyridostigmine by the oral or intravenous route and were evaluated thereafter for inhibition of cholinesterase activity and for morphological abnormalities of the neuromuscular junction. The latter was evaluated by electron microscopy. In addition, the stress of muscle fatigue, as induced by swimming, on pyridostigmine effects was evaluated.

Perturbations of the ultrastructure of the neuromuscular junction, as indicated by excessive swelling of pre- and post-synaptic mitochondria and other post-synaptic organelles, and disruption of Z bands, were observed within 24 hours of oral administration of 12.5 mg/kg pyridostigmine. No such abnormalities were observed after intravenous dosing (at

up to 0.15 mg/kg), and the ultrastructural changes had reversed within 28 days of oral drug administration. Muscle fatigue induced by swimming was not shown to markedly increase the adverse effects of pyridostigmine within the design of these experiments.

Based on these data it was concluded that pyridostigmine administration can cause an acute alteration in the ultrastructure of the neuromuscular junction of the rat. However, the condition is reversed within 28 days.

G-8740-2800 A 14-Day Pilot Oral Gavage Toxicity Study
with Pyridostigmine in Sprague-Dawley Rats

Male and female rats received oral (gavage) doses of 0 (vehicle), 4, 8, 16, 32 or 64 mg/kg pyridostigmine bromide for 14 consecutive days. Clinical signs of pyridostigmine toxicity, including ocular and nasal discharges, tremors, salivation, ataxia, prostration and diarrhea, were observed at 32 and 64 mg/kg, while body weight gains were reduced at 16, 32 and 64 mg/kg. Food consumption was reduced early in the study, but appeared to recover during the second week of treatment. Plasma cholinesterase activity was decreased to approximately 50 percent of control at 4 mg/kg, to approximately 37-50 percent at 8 mg/kg, and to approximately 10-34 percent at 16, 32, and 64 mg/kg. The clinical signs of toxicity appeared to lessen during the second week of treatment, suggesting tolerance development, but plasma cholinesterase inhibition remained relatively constant.

G-8740-3200 Fourteen-Day Pilot Dose Range
Oral Toxicity Study in Dogs

Male and female beagle dogs received oral doses of 0 (vehicle), 5, 10 or 20 mg/day of pyridostigmine bromide for 14 consecutive days. The animals were monitored closely for clinical signs of intoxication and blood samples were obtained on days 1, 7 and 14 for analysis of acetylcholinesterase inhibition. Three of the 4 dogs at 20/mg/kg/day died between the second and the sixth day of drug administration due to an apparent pyridostigmine-induced intussusception. Other signs of anticholinesterase

toxicity were observed at all three pyridostigmine doses and included hypersalivation, lacrimation, tremors, convulsions, ataxia, diarrhea, and emesis. The diarrhea and lacrimation were observed continuously during the study, while the other signs were largely transient, and had reversed prior to the next daily dose. Both plasma cholinesterase and red blood cell acetylcholinesterase activities were decreased in a dose-related fashion. At necropsy, areas of redness and ulceration were observed in the small intestine of pyridostigmine-treated dogs.

G-8740-3300 In Vitro and In Vivo Effects of Pyridostigmine Bromide on Dog Red Blood Cell Acetylcholinesterase Activity

Red blood cell (RBC) acetylcholinesterase activity (AChE) was monitored after oral administration of 0.05, 0.5 or 2.0 mg/kg pyridostigmine bromide to beagle dogs of both sexes. In addition, some dogs received three doses of 0.5 mg/kg pyridostigmine bromide, one dose every eight hours.

Maximum inhibition of RBC AChE occurred 2-6 hours after administration, and was proportional to dose (approximately 8 percent at 0.05 mg/kg, 57 percent at 0.5 mg/kg, and 83 percent at 2 mg/kg). The kinetics of inhibition, and the maximum inhibition, appeared to be the same after the third as after the initial administration of 0.5 mg/kg. Clinical signs of anticholinesterase toxicity (tremors, muscle fasciculations, emesis, diarrhea) were observed at the 0.5 and 2.0 mg/kg doses.

Samples of blood from naive dogs were incubated with pyridostigmine in vitro to evaluate the kinetics of RBC AChE inhibition and recovery. The results indicated a rather slow rate of inhibition, with a maximum at 2 hours of incubation, and little or no change in the extent of inhibition for the subsequent 4.5 hours (the experiment was stopped after 6.5 hours because of increasing hemolysis). Washing of the RBCs enhanced recovery of enzyme activity, suggesting that an excess of uncomplexed pyridostigmine in the blood acted as a reservoir to replace the hydrolyzed drug.

G-8740-3400 Pilot Repeated Oral Dose Study in Dogs to Examine Pyridostigmine Inhibition of Red Blood Cell Acetylcholinesterase

Pyridostigmine bromide was administered once every 8 hours by gavage to male and female dogs for 28 continuous days at doses of 0 (vehicle), 2 or 5 mg/kg. All dogs survived these doses, but signs of severe gastrointestinal distress were observed. Levels of red blood cell (RBC) acetylcholinesterase (AChE) inhibition 8 hours after pyridostigmine administration (just prior to the next oral dose) were approximately 63 percent and 74 percent in males and females, respectively, at 2 mg/kg; and 79 and 84 percent in males and females, respectively, at 5 mg/kg. The degree of inhibition did not change markedly between the initial and the 28th day of administration. Recovery of enzyme activity after cessation of pyridostigmine administration was rapid, with return to approximate basal activities within 72 hours.

These data indicate that the 2 and 5 mg/kg doses of pyridostigmine bromide given orally every 8 hours produced marked inhibition of RBC AChE activity, with little evidence of accommodation, and that recovery was rapid upon removal of the drug.

G-8740-3500 Acute Oral Toxicity Study of WR238605, Succinate in Fischer 344 Rats

Male and female rats received single oral (gavage) administrations of WR238605 over a dose range of 215-935 mg/kg and were observed for the next 14 days. Clinical signs of intoxication included lethargy, nasal discharge, and dyspnea. Doses in excess of 450 mg/kg retarded weight gain or caused weight loss within the first week after treatment. Deaths occurred at all doses in females, and at doses of 275 mg/kg and greater in males; time to death was 2-8 days, with many occurring at 5-6 days. The LD50 values and 95 percent confidence limits for males and females were 476 (382, 612) mg/kg and 528 (357, 1034) mg/kg, respectively.

G-8740-3600 90-Day Oral Dose Toxicity Study
of Pyridostigmine in Dogs

Male and female beagle dogs received pyridostigmine bromide orally (capsule) at doses of 0 (vehicle), 0.05, 0.5 or 2.0 mg/kg every 8 hours for approximately 90 days. Subsets of the dogs receiving 0, 0.5 and 2.0 mg/kg/8 hours were held for a subsequent 90 day untreated recovery period before sacrifice.

The 2.0 mg/kg/8 hour dose caused gastrointestinal distress and a 70-75 percent inhibition of red blood cell (RBC) acetylcholinesterase (AChE) activity. One female dog at this dose developed an apparent drug-induced intussusception and had to be terminated. Both the gastrointestinal distress and RBC AChE inhibition reversed upon discontinuation of the drug. Gastrointestinal distress was less frequent at 0.5 mg/kg/8 hour, and the level of inhibition of RBC AChE was approximately 45-50 percent.

A low incidence of gastrointestinal distress was observed even at 0.05 mg/kg/8 hour, and a slight but consistent inhibition of approximately 5-10 percent of RBC AChE activity was measured.

These data indicate that 0.05 mg/kg/8 hour is near a no-observed toxic effect dose for oral pyridostigmine, while both 0.5 and 2.0 mg/kg/8 hour cause prominent gastrointestinal distress and marked inhibition of RBC AChE activity.

CONCLUSIONS

A large number of studies were conducted under this contract in support of drug development activities for the USAMRDC. These included principally preclinical safety evaluations in support of INDAs to the U.S. Food and Drug Administration. In particular, two new drugs, WR238605, Succinate, an antimalarial, and pyridostigmine, a possible prophylactic drug against organophosphate nerve agent intoxication, were evaluated in acute, subacute, and subchronic studies in dogs, rodents, rabbits, and guinea pigs.

WR238605, Succinate, was shown to be a relatively toxic drug with adverse effects on the hematopoietic system. Pyridostigmine was an effective

inhibitor of red blood cell acetylcholinesterase, but its primary manifestations of toxicity after oral administration was gastrointestinal distress, perhaps reflecting its relatively poor absorbability from the gut and local anticholinesterase activity.

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