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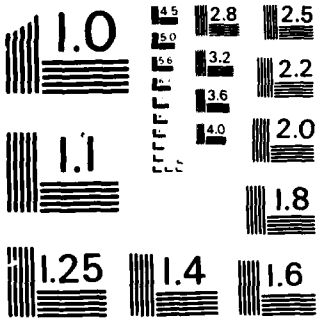

Report

PRELIMINARY TOXICOLOGY OF NEW DRUGS
Report 3740 to 2

to

UNITED STATES ARMY MEDICAL RESEARCH
AND DEVELOPMENT COMMAND
Fort Detrick
Frederick, Maryland 21701-5012

April 4, 1986

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ANNUAL REPORT
Contract No. DAMD17-84-C-4088

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April 4, 1986

by

J. G. Page, Ph.D., D.A.B.T.
Principal Investigator

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Preclinical toxicology studies of WR238605,Succinate and Pyridostigmine Bromide are being performed to assist the U.S. Army Medical R&D Command (USAMRDC) in its decision of whether or not to allow widespread clinical use of these agents. Since these studies provide support for clinical trials in man, they are being conducted in strict compliance with the U.S. Food and Drug Administration's Good Laboratory Practice Regulations (21 CFR Part 58).

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Subacute (28 day) toxicity studies of WR238605,Succinate, a potential antimalarial agent, were conducted in rats and dogs. Toxicity studies to define the reversibility of pyridostigmine-induced changes in the morphology of the neuromuscular junction were conducted in rats. Additionally, subacute (14 day) toxicity studies of pyridostigmine, a reversible anticholinesterase inhibitor, were conducted in rats and dogs. Results of these studies are detailed in individual study reports. Briefly, oral doses of WR238605,Succinate produced methemoglobin formation. Single oral doses (0.1XLD50) of pyridostigmine induced morphological changes in the neuromuscular junctions of diaphragm muscle in the rat. These changes were apparent on Day 1 at one hour after dosing, but were not detected at 28 or 56 days after dosing. Tolerance developed to the clinical signs of toxicity in rats given daily doses of pyridostigmine. Signs of severe gastrointestinal hemorrhage were noted in dogs given daily oral doses of 10 or 20 mg/kg of pyridostigmine. Four dogs died after receiving 10 or 20 mg/kg/day of pyridostigmine. The cause of death for three of the dogs was determined to be intussusception.

All studies are progressing satisfactorily as the contract enters the third year.

ABSTRACT

Preclinical toxicology studies of WR238605,Succinate and Pyridostigmine Bromide are being performed to assist the U.S. Army Medical R&D Command (USAMRDC) in its decision of whether or not to allow widespread clinical use of these agents. Since these studies provide support for clinical trials in man, they are being conducted in strict compliance with the U.S. Food and Drug Administration's Good Laboratory Practice Regulations (21 CFR Part 58).

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All studies are progressing satisfactorily as the contract enters the third year.

FORWARD

These studies have been conducted at Battelle's Columbus Laboratories, utilizing personnel and facilities primarily from the Toxicology and Health Sciences, Pathology and Laboratory Animal Care Sections of the Toxicology Department. The materials contained herein constitute an Annual Report for the second contract year, and cover the period of time from March 1, 1985 - February 28, 1986. The Principal Investigator for these studies was John G. Page, Ph.D., D.A.B.T.

Citations of commercial organizations and 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 Care and Use of Laboratory Animals" prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animals Resources, National Research Council (DHEW Publication No. (NIH) 78-23, (Revised, 1985).

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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 U.S. 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 the regulatory responsibility for approval of new drugs intended for use in humans or animals. 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 animal species is uniquely predictive of human response, many different types of toxicology studies may be required before FDA approval. The number and types of toxicology studies that may be required for FDA approval are dependent upon factors such as the number of people that may eventually be treated with the drug, the length of time that the drug will be used for

treatment of a patient and similarity between the potential therapeutic and compounds with similar chemical structure and of known toxicity that may already be in clinical use.

Upon completion of the critical preclinical toxicology studies (i.e. acute and subacute studies), USAMRDC will file a Notice of Claimed Exemption for an Investigational New Drug (IND) with the FDA. The IND application will contain reports of all preclinical toxicology studies, and other relevant information including chemistry and pharmacology information. Should the FDA decide that the potential benefits of the proposed new drug justify the risks, as might be reasonably expected from extrapolation of preclinical toxicology studies, limited clinical trials will be approved. More focused and longer term toxicity studies may be required to run concurrently with the initial clinical trials in man. Results from these additional animal toxicity studies will be used to asses the potential for chronic toxicity or irreversible effects in humans. Upon completion of the clinical trials in humans and the additional toxicity studies in animals, all relevant data will be filed with the FDA in the form of a New Drug Application (NDA). If the FDA approves the NDA, the new therapeutic can be introduced into general clinical use.

The importance of preclinical toxicology studies in the development and use of potential therapeutics demands that these studies be conducted by a reputable organization with a competent and experienced staff and adequate facilities. The FDA recognized the importance of preclinical toxicology studies, and issued its Good Laboratory Practice (GLP) regulations (21 CFR Par. 58) to govern the proper conduct and reporting of preclinical toxicology studies. Each of the studies described in this report has been conducted in compliance with the FDA GLP Regulations.

RESULTS AND DISCUSSION

A number of studies have been initiated under this contract during the second year. The status of each of these studies is shown in Table 1. Table 2 lists studies that are currently anticipated to start during the third contract year. Projected start dates are based upon information available on February 28, 1986. Table 3 contains a list of quarterly and annual reports for the third contract year.

TABLE 1. STATUS OF STUDIES INITIATED DURING THE SECOND CONTRACT YEAR

Study No.	Title	Status
G8740-2300	28 Day Subacute Dog WR238605,Succinate	Draft Study Report Submitted
G8740-2500	28 Day Subacute Rat WR238605,Succinate	Draft Study Report Submitted
G8740-2700	Neuromuscular Junction/Muscle Activity Study-Rat Pyridostigmine	Draft Study Report in Preparation
G8740-2800	14 Day Subacute Rat Pyridostigmine	Draft Study Report Submitted
G8740-3200	14 Day Subacute Dog Pyridostigmine	Draft Study Report Submitted

TABLE 2.

Study No.	Title	Anticipated Start Date
G8740-3300	Preliminary Dog Toxicity Study; Pyridostigmine	April, 1986
G8740-3400	Six Month Dog Toxicity Study; Pyridostigmine	May, 1986
Not Assigned	Preliminary Rat Toxicity Study; Pyridostigmine	May, 1986
Not Assigned	Six Month Rat Toxicity Study; Pyridostigmine	June, 1986
Not Assigned	Two Year Rat Chronicle/Carcinogenesis Study; Halofanthrin	June, 1986
Not Assigned	Eighteen Month Mouse Chronic/ Carcinogenesis Study; Halofanthrin	June, 1986
Not Assigned	One Year Dog Chronic Toxicity Study; Halofanthrin	July, 1986

TABLE 3. SCHEDULE OF REPORTS FOR THIRD CONTRACT YEAR

Report	Due Date
First Quarterly	June 21, 1986
Second Quarterly	September 21,1986
Third Quarterly	December 21, 1986
Fourth Quarterly	March 21, 1987
Third Annual	March 31, 1987

The status and results of each study are described briefly in the following paragraphs.

G8740-2300 28 Day Oral Toxicity Study with WR238605,Succinate in Beagle Dogs

WR238605,Succinate was suspended in a methylcellulose/Tween 80 vehicle, and doses of the resulting suspension administered in gelatin capsules to dogs, each day for 28 days. Doses of 3.0 to 15.7 mg/kg/day of the succinate salt were tested. A treatment-related cyanotic appearance of external tissues (i.e. ear flaps, nose, muzzle) was detected in most treated dogs during the latter three weeks of this study. The cyanotic appearance was attributed to methemoglobin formation. There was a treatment-related decrease in red blood cells and platelets, but only slight increase in reticulocytes. Significant treatment-related increases were noted in the liver weights of male dogs and the spleen weights in female dogs. Drug-related lesions were detected in the lung, liver and lymphoid tissues. A no-effect dose was not demonstrated in this study.

G8740-2500 28 Day Oral Toxicity Study of WR238605,Succinate in Fischer 344 Rats

Suspension of WR238605,Succinate in a methylcellulose/Tween 80 vehicle were administered daily by oral gavage to young adult male and female Fischer 344 rats. Doses of 3.8 to 38.5 mg/kg/day of the succinate salt were evaluated. One group was given daily doses of 34.5 mg/kg/day for 28 days and held for an additional 14 days (drug free) to assess reversibility of drug-induced toxicity. Clinical signs of toxicity included pallor, ruffled fur, abnormal posture, yellow staining of the urogenital area (females only) and thin appearance. No mortalities occurred. Treatment-related decreases in food consumption and body weight gain were noted. Increases in SGOT, SGPT, serum glucose, methemoglobin, reticulocytes and in RBC's were noted in WR238605,Succinate-treated animals. Liver and spleen weights increased in treated animals, while treatment-related decreases were recorded for brain, heart and gonad weights. Pulmonary edema and aspermatogenesis were noted in

treated animals. A no effect dose was not demonstrated in this study. Reversibility of WR238605,Succinate-induced toxicity would not be assessed in this study since some evidence of toxicity (e.g. microscopic lesions) were noted in rats in the recovery group.

G8740-2600
G8740-2700 Extent and Reversibility of Acute Effects of Pyridostigmine Including Neuromuscular Junction Lesions

Acute oral and intravenous LD50 values were estimated from mortality data that were obtained in an toxicity study with pyridostigmine bromide (Mestinon•, Roche). The effects of pyridostigmine on a number of enzymes (CPK, CPK isoenzymes, LDH, pyruvate kinase and plasma pseudocholinesterase) were examined at various times after single oral or intravenous doses of Mestinon• to Sprague Dawley rats. Samples of diaphragm, EDL muscle, soleus muscle and heart (A-V node) were collected from nonexercised rats (G8740-2600) and exercised rats (G8740-2700) that had been given single oral or intravenous doses of Mestinon•. Samples of diaphragm muscle were taken from selected rats and examined with the aid of an electron microscope. Pyridostigmine-induced lesions were noted one hour after single oral doses of Mestinon•, but no drug-related lesions were noted after intravenous doses of Mestinon•. Drug-related lesions noted in the oral dosed animals were not apparent on Days 28 or 56 of the study. These findings suggested that pyridostigmine-induced lesions were reversible. A draft report should be available in April, 1986.

G8740-2800 A 14 Day Pilot Oral Gavage Toxicity Study with Pyridostymine in Sprague Dawley Rats

Male and female Sprague Dawley rats were given oral gavage doses of 0-64 mg of pyridostigmine bromide/kg/day for 14 days. Data from this study will be used to set doses and develop a study design for a subchronic (i.e., 6 month) toxicity study in rats. Pyridostigmine-induced signs of toxicity included ocular discharge, nasal discharge, hypoactivity, prostration, ataxia, diarrhea, hunched posture, thin appearance and death. Clinical signs

decreased in incidence and severity during the latter half of the study. Dose-related decreases in body weight and food consumption were noted during this 14 day study. Leukocyte counts were suppressed in male and female rats in the high dose (i.e. 64 mg/kg/day) group. Serum glucose and alkaline phosphatase levels were increased, while SGOT, LDH, CPK and pyruvate kinase levels decreased at 24 hours after the final dose of pyridostigmine. Plasma pseudocholinesterase levels were inhibited at pyridostigmine doses of 4-64/mg/kg/day with maximum inhibition noted at doses of 16-64 mg/kg/day. Some recovery in plasma pseudocholinesterase activity was noted in rats receiving suboptimum (i.e., 4-8 mg/kg/day) doses of pyridostigmine. Results indicated that tolerance developed to the pyridostigmine-induced toxicity during the course of this study.

G8740-3200 A 14 Day Pilot Dose Range Oral Toxicity Study in Dogs with Pyridostigmine

Oral doses of pyridostigmine bromide, 5 to 20 mg/kg/day, were administered daily to beagle dogs. Most of the clinical signs of toxicity that were noted during this study were attributed to the cholenergic effects of pyridostigmine. Clinical signs were noted during each day of dosing, but few effects were noted in the mornings prior to dosing, which suggested a lack of cumulative toxicity. Four dogs died during the 14 day study, and death in three of these dogs was attributed to intussusception. Some evidence of hemoconcentration was noted in these four dogs prior to death. Large increases in numbers of leukocytes reflected increases in concentrations of segmented neutrophils. Pyridostigmine inhibited plasma pseudocholinesterase, with no apparent difference in the level of inhibition at any of the three doses tested. It was concluded that maximum inhibition of plasma pseudocholinesterase occurred at daily doses of 5 mg/kg/day. A no effect dose was not determined in this study.

Other Relevant Information

Previously, studies with pyridostigmine conducted under this contract, utilized a radiometric method to measure the levels of whole blood and plasma cholinesterase. Red blood cell (RBC) acetylcholinesterase was calculated as the difference between these two values. Current plans include development of a spectrophotometric assay to measure RBC acetylcholinesterase activity directly. The assay will be validated in April, 1986, and will be used in all future studies with pyridostigmine.

CONCLUSIONS

A number of toxicity studies were conducted during this contract year. WR238605,Succinate a potential antimalarial agent, and pyridostigmine bromide, a reversible acetylcholinesterase inhibitor were tested in toxicity studies in rats and dogs. Battelle staff have received a great deal of scientific satisfaction from working with LTC William E. Ridder, the C.O.T.R., and other USAMRDC personnel on these projects, and they look forward to the continuation of these studies and implementation of studies with other compounds during the third contract year. Further discussion regarding the significance of study results will be reserved for individual study reports and for the final, inclusive contract report.

BIBLIOGRAPHY

1. 28 Day Oral Toxicity Study with WR238605,Succinate in the Beagle Dog. Study No. G8740-2300. J. G. Page and D. C. Emmerling. Battelle Columbus Laboratories. November, 1985 (Report 8740-85-18).

2. 28 Day Oral Toxicity Study with WR238605,Succinate in Fischer 344 Rats. Study No. 8740-2500. J. G. Page and D. C. Emmerling Battelle Columbus Laboratories. September, 1985. (Report No. 8740-85-15).

3. Single Dose LD50, Enzyme and Pathology Study with Pyridostigmine in Sprague Dawley Rats. Study No. 8740-2600. J. G. Page. In preparation, March, 1986.

4. Muscle Activity Study with Pyridostigmine in Sprague Dawley Rats. Study No. G8740-2700. J. G. Page. In preparation, March, 1986.

5. A 14 Day Pilot Oral Gavage Toxicity Study with Pyridostigmine in Sprague Dawley Rats. Study No. G8740-2800. J. G. Page and D. C. Emmerling. September, 1985. (Report No.8740-85-15).

6. A 14 Day Pilot Dose Range Oral Toxicity Study in Dogs with Pyridostigmine. Study No. G8740-3200. J. G. Page and D. C. Emmerling. November, 1985. (Report No. 8740-85-16).

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