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FUNCTIONAL CARDIORESPIRATORY TOXICITY SCREENING OF CANDIDATE ANTIPARASITIC DRUGS AND ANTIDOTES FOR CHEMICAL POISONS

Subtitle: STUDY OF THE EFFECTS OF DRUGS UPON THE CARDIOVASCULAR AND RESPIRATORY SYSTEMS

ANNUAL REPORT

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During this past year we have 1. completed experimental work on the Effects of Chloroquine and Mefloquine Individually in Combination Upon Automaticity, Rhythmicity and Dynamics of the Heart. Summary attached. 2. Written a protocol to study the Effects of Mefloquine and Pyridostigmine Individually and in Combination Upon Cardiac Automaticity. A copy of this protocol is attached.

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SUMMARY

1. Completed experimental work on the Effects of Chloroquine and Mefloquine Individually and in Combination Upon Automaticity, Rhythmicity, and Dynamics of the Heart. Summary is attached.

2. During this past year we have: written a protocol to study The Effects of Mefloquine and pyridostigmine Individually and in Combination Upon Cardiac Automaticity. A copy of this protocol (submitted on 18 September 1987) is attached.

FOREWORD

In conducting research using animals, the investigator(s) 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 (NIH Publication No. 86-23, Revised 1985).

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SUMMARY

Twenty-four adult beagle dogs anesthetized with pentobarbital Na were injected with formalin into the A-V node and adjoining bundle of His to attain complete heart block. This was done in order to observe separately the actions of chloroquine and mefloquine on the automaticity of both the atria and the ventricles. Chloroquine and mefloquine are both antimalarial drugs which are known to interfere with normal cardiac impulse formation and contractility. Since the possibility exists that mefloquine may be administered following chloroquine treatment where resistance to chloroquine has arisen, it was deemed necessary to test the two drugs in combination to determine if any antagonism, additivity, or potentiation of effects on cardiac automaticity (rhythm) occur.

Thirty minutes after inducing heart block, baseline measurements were taken for intrinsic rates for both atria and ventricles, arterial blood pressure, and left ventricular pressure. Following these measurements, the atria and ventricles were simultaneously overdriven electrically for 2 minutes using square wave DC pulses of 5 msec duration at a voltage 3 times the driving threshold. The atria were driven at 200 beats/minute and the ventricles at 150 beats/minute. These frequencies represent overdrive values considering the normal intrinsic rates.

Immediately following overdrive, simultaneous assessments of atrial and ventricular automaticity were made by measuring the period of asystole (period following cessation of stimulation until first depolarization), the period for the first 10 depolarizations, and the number of depolarizations in the first 30 second period.

Section I

After drug administration, this overdrive process was repeated and measurements taken every 10 minutes up to 100 minutes. The drugs given were either the control (5% dextrose in water -D5W), ED_{50} chloroquine in D5W, ED_{50} mefloquine in D5W, or a combination of $\frac{1}{2}$ ED_{50} chloroquine + $\frac{1}{2}$ ED_{50} mefloquine in D5W.

It was found that four of our experimental variables exhibited responses to chloroquine, mefloquine or the drug combination. Intrinsic atrial rate and the number of atrial beats in 30 sec following overdrive were significantly depressed by all three treatments. Left ventricular dp/dt was depressed only by chloroquine; no other treatments affected dp/dt. For each of these variables, there were no differences among the treatment groups, only differences between treatment groups and the control group. For all other variables, neither of the drugs alone nor the combination produced any discernable effects.

In the case of all measured variables, the values or responses for the combination treatment group were not different from those in the groups given mefloquine or chloroquine alone. Therefore, by Gaddum's definitions of possible drug interactions, only simple addition of effects occurred when mefloquine and chloroquine were combined.

Section II

INTRODUCTION

Mefloquine is an antimalarial drug that is known to interfere with normal cardiac impulses and contractility (Arora and Lai, 1963; Hemwell and Di Palma, 1979; Caldwell and Nash, 1977). Pyridostigmine bromide is a reversible inhibitor of acetylcholinesterase activity which increases the plasma and tissue half-life of acetylcholine. The resulting biological effect of pyridostigmine upon the cardiovascular system is reduced heart rate (Caldwell, et al., 1986). Preliminary studies in our laboratory have shown the mefloquine reduced the automaticity of the ventricle but not that of the atria. On the other hand, pyridostigmine decreased automaticity in the atria, but not in the ventricle (Caldwell, et al., 1986). The possibility exists that pyridostigmine may be administered following treatment with mefloquine, therefore any combined effects that may exist need to be revealed.

PURPOSE

To determine whether a combination of these drugs will augment, antagonize, or have no influences upon cardiovascular variables as compared to each drug independently administered.

EXPERIMENTAL PREPARATION

The dogs used in this study were divided into four groups of six each as follows:

1. Mefloquine - 8 mg/kg
2. Pyridostigmine - 2 mg/kg
3. Combination - Mefloquine (4 mg/kg) - pyridostigmine (1 mg/kg)
4. Vehicle control

Pure bred beagle dogs of either six, 9 months and older, weighing between 9.0 and 14.0 kg will be purchased from Riglan Animal Care Systems (Mt. Horan, WI). This company guarantees that all dogs will be in excellent

health upon arrival at the Medical College of Georgia Vivarium. The dogs will be kept in quarantine and under the care of Medical College of Georgia veterinarians who will verify their excellent health upon receipt and thereafter.

Sodium pentobarbital, 30 mg/kg intravenously, will be used to anesthetize the dogs. Supplemental doses will be administered as needed to maintain proper anesthesia.

A T-shaped cannula will be inserted into the trachea and the dog will be placed on a Harvard respirator breathing room air at 25 ml/kg tidal volume at a rate of 10-15 breaths/min.

A femoral artery will be catheterized with polyethylene tubing filled with heparinized saline advanced to the thoracic aorta for measurement of arterial blood pressure via a Statham P23AC pressure transducer.

One cephalic vein will be catheterized for administration of drug. In experiments where two drugs are given, both cephalic veins will be used and the two drugs infused simultaneously.

The heart will be exposed via a mid-sternal chest incision and the pericardium removed. Two small plexiglass plates (1.5 x 0.5 cm) each having four platinum electrodes will be sewn to a convenient site upon the surface of the right ventricle and right atrial appendage to provide good contact. Two electrodes from each plate will be used to record the electrogram. The two remaining electrodes will be used to drive the atria and ventricles at separate rates using two Grass stimulators, model SD9. The electrograms from the atrium and the ventricle will be recorded on a Grass Polygraph along with the blood pressure.

In a previous study (Caldwell and Nash, 1977) it was determined that a dose of 8 mg/kg of mefloquine gave cardiovascular responses near the middle of the dose-response curves. This dose produced a reduction in automaticity

which occurred only in the ventricles, and no apparent change occurred in the atria.

A dose of 5 mg/kg of pyridostigmine was determined to reduce heart rate and acetylcholinesterase activity to a point just short of death (Caldwell, et al., 1986). A dose of 2 mg/kg produced intermediate responses and was, therefore, used as a basis for our preliminary studies of automaticity. Experiments on automaticity using 2 mg/kg of pyridostigmine did show reduced automaticity in the atria.

Drug Preparation and Administration: Mefloquine will be supplied by WRAIR along with an assay report. Mefloquine is water soluble to some degree (2 mg/ml) and will be dissolved and administered in 5% dextrose in water. Adequate supplies of pyridostigmine in the form of Mestinon® will be furnished by WRAIR along with an assay report. Mestinon® is in liquid form and will be diluted in 5% dextrose in water. Both drugs will be administered intravenously via a Cole-Palmer variable speed pump in a constant volume of 4 ml/kg over 10 minutes.

A-V Conduction blockade: Complete heart block by the method of Steiner and Kovalik (1968) will be achieved by injecting 0.1 ml of 40% formaldehyde into the atrial septum at the level of the A-V node and the adjoining common bundle of His. Injection will be accomplished via a 25 gauge needle placed at a depth of 0.5 to 1.0 cm below the groove between the atrium and aorta. Complete heart block is verified by lead II ECG recording.

Procedure: Our procedure is patterned after Afonso et al., (1972) and Korte and Nash (1978). Following surgery and A-V block, the dog will be allowed to equilibrate for approximately 30 minutes.

The atria and ventricles will be simultaneously overdriven for 2 minutes with a square wave D.C. pulse of 5 msec duration at a voltage strength 3 times the driving threshold (Korte and Nash, 1978). Threshold

will be determined by the minimum voltage required to drive the atria and ventricles. We will drive the atria at 200 beats/min and the ventricles at 150 beats/min. Immediately following the period of overdrive, assessment of automaticity will be made as follows: (or the atria and ventricles independently).

1. The time required for the first 10 beats following cessation of stimulation.

2. The number of beats that occur in the 30 second period following the cessation of stimulation.

3. Asystole period in seconds following cessation of stimulation until first heart beat.

In addition to the above measurements, we will record intrinsic heart rate and systolic and diastolic blood pressure.

Observation period: Baseline values will be taken at -30 min. and 0 time before infusing the drug. The experiment will continue for 3 hours following drug administration with assessments of automaticity taken every 30 mirutes. Prior to each overdrive period, the intrinsic heart rates of both the ventricles and atria will be determined along with arterial blood pressure.

Analysis of data: Each of the four experimental groups will be comprised of six dogs. Following all necessary experiments, the value of each time point for each individual in each treatment group will be plotted over the 180 minute period of the experiment. The data over the observation period for each dog will then be averaged and divided by the mean of the respective baseline (zero time) to obtain the change in response ratio following treatment. The ratios for each variable from each treatment will be plotted as a linear horizontal sensitivity graphic. These plots will furnish a visual impression of the treatment effects. The statistical

analysis will involve a one-way analysis of variance for significance within the overall experiment, followed by use of the Newman-Keuls technique to establish which changes are significant (Winer, 1971).

This data treatment will help us to decide which of the following possible interactions may have occurred:

1. Potentiation (0.5 A + 0.5 B > the larger of A or B)
2. Antagonism (0.5 A + 0.5 B < the smaller of A or B)
3. Simple Addition)0.5 A + 0.5 B = A or B, or a value between A and B)

Upon completion of a test, each dog shall be euthanized using an over-dose of pentobarbital; monitoring shall be continued until cardiac and respiratory standstill have been observed.

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1. The conduct of these studies shall comply with the GOOD LABORATORY PRACTICES (GLP) regulations as published in the Federal Register, Volume 43 (247), 22 December 1978, Part II, pp 59, 986-60,020 (and all subsequent addenda).

2. In the proposed studies, the investigators will adhere to the principles outlined in the current "Guide for the Care and Use of Laboratory Animals", Public Health Service National Institute of Health, NIH Publication No. 85-23, Revised 1985.

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