

**PHARMACOKINETICS AND DOSE RANGE-FINDING
OF
IN BEAGLE DOGS**

SPONSOR: Toxicology & Pharmacology Branch
Developmental Therapeutics Program
Division of Cancer Treatment, Diagnosis, and Centers
National Cancer Institute
National Institutes of Health
Bethesda, Maryland 20892

PROJECT OFFICER:

CONTRACT NUMBER:

CONTRACTOR:

PRINCIPAL INVESTIGATOR:

STUDY DIRECTOR:

PROPOSED IN-LIFE PHASE:

Start:

Finish:

I. OBJECTIVE

The objective of this study is to determine plasma elimination kinetics of _____ and to evaluate toxicity after a single intravenous or oral administration of _____ in dogs.

II. MATERIALS AND METHODS

A. Test and Control Articles:

1. Name of Test Articles:

2. Name of Control Articles:

3. Characterization and Documentation of Methods of Synthesis, Fabrication or Derivation:

a. Test Article:

Compound identity, strength, quality, stability and purity as well as documentation of methods of synthesis, fabrication or derivation are the responsibility of the NCI. Confirmation of identity will be done immediately upon receipt of each shipment of the compound. Sufficient quantity of drug will be reserved for archiving from each lot and shipment used.

b. Control Article:

4. Stability and Storage:

a. Test Article:

b. Control Article:

5. Formulation, Preparation, Stability and Storage:

6. Dose Concentration and Homogeneity Analyses:

Homogeneity analysis will be done on a randomly selected sample. Homogeneity analysis will consist of dose concentration analysis of a top, middle and bottom portion of the selected sample. Dosing solutions must be within 10% of the theoretical concentration and all results of these analyses will be submitted to the NCI COTR within 7 days after each dosing. An adequate quantity of each dosing mixture will be retained for possible rDose concentration analyses until the acceptance of the final report on this compound.

7. Compatibility and Hemolytic Potential Testing of Dosage Formulation:

These tests are to be initiated before initial compound dosing of dogs according to the procedures in Appendix A.

B. Test System:

1. Species, Strain Supplier and Test System Justification:

Purebred beagle dogs will be used in this study. This is an accepted species to support studies of compounds used or intended for use in humans.

2. Initial Age, Sex and Weight:

Male and female dogs will be approximately 8 to 12 months of age and approximately 7 to 14 kg at study initiation.

3. Care and Housing:

General procedures for animal care and housing will be in accordance with DHHS Publication No. (NIH) 86-23 (Revised, 1985) and the U.S. Department of Agriculture through the Animal Welfare Act (7 USC 2131), 1985 and Animal Welfare; Standards incorporated in 9 CFR Part 3, 1991. The dogs will be housed individually in stainless steel cages.

4. Diet and Water Supply:

A certified, commercial, dry chow or meal with the following minimum composition will be used:

- Approximately 10% moisture
- At least 20% crude protein
- Approximately 5% fat
- Nutritionally adequate amounts of minerals
- Both water soluble and fat soluble vitamins

Dogs will have exposure to their daily ration for a total period of 1 to 2 hours per day. The quantity of the daily ration will be sufficient to meet nutritional requirements. Water source will be the public supply given *ad libitum*. No contaminants will be present in the feed or water which could interfere and affect the results of the study.

5. Quarantine:

Dogs will be quarantined for a minimum of 14 days prior to baseline measurements. A complete physical examination including a fecal examination for internal parasites, clinical pathology, body weight and rectal temperature will be performed on dogs within 7 days of delivery. See Section II.C.4.c. for clinical pathology. All data will be recorded. If the physical examination indicates the presence of internal parasites, dogs will be administered a vermifuge approved by the NCI Project Officer. Only positive dogs will be treated for parasites. If treatment is necessary, a minimum of 28 days will elapse prior to initial dosing. The dogs selected for study will be in good physical condition.

6. Animal Identification:

Dogs will be uniquely identified by ear tattoo number or letter combination. Positive identification will be required at least after each cage change, blood sampling and dosing.

C. Experimental Design:

1. Group Assignments:

Two groups of two dogs (one male and one female per group) will be dosed as follows:

GROUP	DOSE (mg/kg)
I	TBD
II	TBD

2. Route of Administration and Reason for Choice:

The test article will be given intravenously because this is the intended route of administration of this compound in humans.

3. Dosing Procedure:

The test article will be administered by a single intravenous injection on day 1.

Dose calculations will be based on the individual body weight on the treatment day. All calculations for amount of drug to each dog will be checked by a second individual who will initial and date the verification.

4. Measurements:

a. Clinical Signs:

PRETEST - Observe dogs daily and record all clinical signs beginning on study day -3. Baseline body temperatures will be taken once on day -3.

TEST - All adverse clinical signs will be recorded. Dogs will be observed for the first three hours after treatment on day 1. At a minimum, observations will be recorded at 1, 3, 6 and 12 hours on the day of treatment and once daily during the remainder of the study or more often as clinical signs warrant.

Body temperatures will be measured and recorded three hours after the dose is administered on study day 1.

b. Body Weight, Food and Water Consumption:

PRETEST - Body weights will be recorded on day -3. Dogs will be fasted overnight before treatment and 6 hours following treatment. Food and water consumption measurements will not be required.

TEST - Body weights will be recorded on study days 1, 8 and 15. Food and water consumption measurements will not be required.

c. Clinical Pathology:

PRETEST - All dogs will be fasted overnight and bled for clinical pathology once on day -3. The baseline sample will be taken after the quarantine period is completed. Dogs that have aberrant values will not be used.

TEST - All dogs will be fasted overnight and blood drawn on days 2, 4, 8 and 15. The procedures will be performed according to the laboratory's SOP but in no case will dogs be bled from the treatment site. A blood sample will be obtained prior to the necropsy of each dog sacrificed in a moribund condition.

Hematology:

Erythrocyte count (RBC) - $10^6/\text{mm}^3$

Hemoglobin (HGB) - g/dL

Hematocrit (HCT) - %

Mean corpuscular volume (MCV) - fl

Mean corpuscular hemoglobin (MCH) - pg

Mean corpuscular hemoglobin concentration (MCHC) - g/dL

Platelet count (Plate) - $10^3/\text{mm}^3$

Reticulocyte count (RETIC) - % RBC

Total leukocyte count (WBC) - $10^3/\text{mm}^3$

Differential leukocyte count - %

Nucleated red blood cell count (nRBC) - nRBC/100 WBC

Clinical Chemistry:

Blood urea nitrogen (BUN) - mg/dL

Serum aspartate aminotransferase (AST) - I.U./L

Serum alanine aminotransferase (ALT) - I.U./L

Alkaline phosphatase (Alk. Phos.) - I.U./L

Serum glucose (BS) - mg/dL

Prothrombin time (PT) - sec

Creatinine (CREAT) - mg/dL

Total protein (T PROTEIN) - g/dL

Sodium (Na) - meq/L

Potassium (K) - meq/L

Chloride (Cl) - meq/L

d. Plasma Drug Level Determination:

Blood samples will be obtained from each dog at the

following times after dosing on day 1:

0, 2, 5, 10, 20, 30, 60, 90, 120 (minutes), 4, 8, 12, 16, 20 and 24 (hours).

An aliquot of each sample (approximately 2 mL) will be mixed with EDTA. The samples will be centrifuged and the plasma frozen at -20C until analyzed. A blood sample will be drawn for plasma drug analysis prior to the sacrifice of moribund dogs. Analytical procedures have been previously supplied in the compound information.

e. Necropsy Procedure:

Dogs will be taken off study on day 15, but will not be sacrificed.

UNSCHEDULED SACRIFICE - Moribund animals will be sacrificed to minimize the degree of postmortem autolysis. The authorization to sacrifice moribund dogs will be made by the Study Director or other qualified individual after examination of the dogs. If a dog is found dead outside of normal working hours, the dog will be necropsied as soon as possible with the carcass refrigerated (not frozen) in the interim period (not to exceed 24 hr). Body weight will be taken and tissues will not be discarded because of postmortem autolysis.

All dogs that die or are sacrificed moribund will have a complete necropsy. Antemortem observations will be recorded for each dog and commented on or confirmed at necropsy. Dogs which are clinically normal will also be indicated. A pathologist will be available to examine any unusual findings.

The tissues listed below will be examined, sampled and fixed in cold, buffered neutral 10% formalin.

- Adrenal glands (2)
- Aorta
- Bone, femoral head with articular surface
- Bone marrow, sternum
- Bone marrow-rib, costochondral junction
- Brain
- Cecum
- Colon
- Duodenum
- Epididymides (2)
- Esophagus
- Eyes (2)
- Gall bladder
- Gonads (2)

Gross lesions
Heart
Ileum
Jejunum
Kidneys (2)
Lip
Liver
Lungs
Lymph nodes (bronchial, mandibular, mesenteric)
Mammary gland (when present in regular abdominal skin section)
Pancreas
Parathyroid gland (when present in regular thyroid gland section)
Pituitary gland
Prostate gland
Salivary gland, mandibular
Sciatic nerve
Skeletal muscle
Skin: 1. ventral abdomen 2. injection site
Spinal cord, thoracolumbar (cervical and posterior lumbar spinal cord examined if nervous system signs present)
Spleen
Stomach (cardiac, fundic, and pyloric)
Thymus
Thyroid glands
Tongue
Tonsils (2)
Trachea
Urinary Bladder
Uterus

The identification mark from the dog will be preserved in fixative.

All fixed tissues will be retained for possible future histopathology evaluation.

f. Microscopic Pathology:

A microscopic evaluation will not be necessary unless directed by the NCI Project Officer. If a necropsy is deemed necessary, tissues will be examined microscopically by a pathologist. Records of gross findings for a specimen from postmortem observations shall be available to the pathologist when examining that specimen histopathologically.

All lesions will be categorized either as drug-related or nondrug related. Each lesion should be listed and coded by the most specific topographic and morphologic diagnoses, severity and distribution using the pathology code tables found at the National

Toxicology Program website
<http://ntp.niehs.nih.gov/?objectid=72016020-BDB7-CEBA-F3E5A7965617C1C1>, may also be used.

Reporting of Pathology data should follow as closely as possible guidelines presented in "Toxicologic Pathology, 34:806–809, 2006"

III. **QUALITY ASSURANCE**

A. **Type of Study**

This is a nonclinical laboratory study and will require compliance with the FDA Good Laboratory Practice Regulations. Data from this study will be included as part of a final report to be submitted to the FDA.

B. **Standard Operating Procedures**

All operations pertaining to this study, unless specifically defined in this protocol, will be performed according to the standard operating procedures of the laboratory and any deviations will be documented.

C. **Protocol Amendments**

All changes in or revisions of an approved protocol and the reasons therefore will be documented, signed, and dated by the Principal Investigator, Study Director and the NCI Project Officer. Amendments will be maintained with the protocol. Verbal approval for a protocol change may be granted by the NCI Project Officer, but a written amendment will follow.

D. **Records**

Data will be verified by the laboratory's Quality Assurance Unit and stored in accordance with the Good Laboratory Practice Regulations.

IV. **REPORTING AND DISCUSSION OF DATA**

A. **Progress Reports**

Status reports summarizing the progress of the study will be provided at monthly intervals. These reports will detail the status of the study on the reporting date, any problems encountered and proposed means of resolution.

B. **Final Report**

The data and results of this study will be submitted as a separate draft report, due 15 working days after the in-life phase of the study is completed. If necropsy occurs, the draft report is due 30 days after the necropsy date. The final report will be due 15 working days after return of the draft report for revision.

This report will accurately and completely describe the study design, procedures and findings, present an analysis and summary of the data followed by the conclusions derived from the analyses. The report will also include: (a) a cover page which will include the title, contract number, authors, laboratory address, dates of initiation and completion, and sponsor; (b) the NTIS Report Documentation Page, to be placed at the beginning of the final report; (c) a comprehensive summary to be placed after the NTIS page; (d) the signature of the Study Director and any others deemed necessary; (e) a table of contents; and (f) a statement prepared and signed by the Quality Assurance Unit which will refer to all phases of the study and where the raw data records, reports and samples are stored.

Protocol Approvals:

Study Director:

(Date)

Principal Investigator:

(Date)

NCI Project Officer:

(Date)

APPENDIX A

Compatibility and Hemolytic Potential Testing of Dosage Formulation:

These tests are to be initiated before initial dosing of dogs.

A. Compatibility:

Mix 1 mL of drug/vehicle (formulated at the highest concentration of drug anticipated) with an equal volume (1 mL) of fresh canine serum. If the combination is incompatible (e.g., if precipitation or coagulation occurs within 30 minutes), repeat the procedure using an equal volume of vehicle alone (1 mL) and serum (1 mL). If the vehicle is incompatible, retest using one-half the concentration of vehicle (dilute with 0.9% sodium chloride, USP, *q.s.* 1 mL) with 1 mL of serum; repeat using two-fold dilutions of vehicle until there is no evidence of vehicle incompatibility. If the vehicle alone does not produce incompatibility, repeat the procedures in which the incompatibility occurred, mixing one-half the concentration of drug in vehicle with serum (keeping the 1 mL to 1 mL volume ratio constant). If this combination is incompatible, decrease the concentration of drug in vehicle by one-half again and mix with an equal volume of serum (1 mL). Continue this procedure until there is no incompatibility. If a greater concentration of drug is later utilized in the dog studies, repeat the compatibility tests using the higher concentration of formulated drug.

Perform the same procedure using fresh canine plasma and fresh human serum

and plasma.

B. Hemolytic Potential:

Using fresh canine blood treated with heparin, set up four tubes containing:

1. A volume of formulated drug with vehicle (at the highest concentration of drug anticipated) and an equal volume of fresh blood.
2. A volume of vehicle and an equal volume of fresh blood.
3. A volume of blood and an equal volume of fresh plasma.
4. A volume of 1 percent saponin (1 g/100 mL 0.9% sodium chloride) and an equal volume of blood as a positive control.

Incubate the four tubes for 45 minutes at 37 degrees C. Centrifuge the tubes (x1000 g) for 5 minutes. Quantitate the amount of hemoglobin in the supernatant plasma using the cyanmethemoglobin method. If the hemolysis of the drug-blood combination is greater than that of the blood plasma control, decrease the concentration of the drug in vehicle by one-half and mix with an equal volume of fresh blood. Continue this procedure decreasing the concentration of drug in vehicle by one-half until the amount of hemolysis is the same as the blood-plasma control. If hemolysis of the vehicle-blood combination is greater than that of the blood-plasma control, decrease the concentration of the vehicle by one-half (dilute with 0.9% sodium chloride (USP), *q.s.* 1 mL) and mix with an equal volume of fresh blood. Continue this procedure decreasing the concentration of vehicle by one-half until the amount of hemolysis is the same as the blood-plasma control.

Perform the same procedures as indicated above for canine blood using fresh human blood treated with heparin.