SINGLE INTRAVENOUS ADMINISTRATION CARDIOVASCULAR SAFETY STUDY OF P1 IN MALE AND FEMALE BEAGLE DOGS

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SRI Study No.: M501-06
SRI Project No.: P17549.200

Study Initiation: November 28, 2006

Experimental Work Performed:
Start: November 29, 2006
Finish: January 25, 2007

Study Completion: April 12, 2007

Sponsor: National Institute of Mental Health, NIH, DHHS NCS Building, Rm. 7185 (MSC 9641) 6001 Executive Blvd. Bethesda, Maryland 20892-9641

Sponsor's Representative: Jamie Driscoll, Program Analyst, NIMH Robert Innis, Ph.D., NIMH

NIMH Contract No. N01-MH-32001, WA#26
Single Intravenous Administration Cardiovascular Safety Study
of P1 in Male and Female Beagle Dogs
SRI Study No. M501-06

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4-12-07  
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SUMMARY

The objective of this study was to determine any potential cardiovascular or toxicity effects of P1 after a single intravenous (IV) injection of 100 or 500 times the proposed human dose level, and to determine a no-observed-adverse-effect-level (NOAEL) in male and female Beagle dogs.

This study involved three (Days 1, 8, and 15) dose administrations of P1 separated by one week intervals. Body weights were collected to determine dose calculations for each dose administration. Cardiovascular (CV) baseline data were collected prior to each dose administration including electrocardiogram (ECG), heart rate, respiratory rate, and blood pressure (indirect). Body temperature was also collected. Following administration of P1, each CV parameter was collected at 2, 30, 60, 120, 240 min, and 24 hr post dose administration. Clinical observations were made immediately post dose and daily thereafter for the duration of the study.

The first IV administration of P1 was 26.43 µg/kg (528.6 µg/m²), 100 times the proposed human dose level. No adverse signs were observed for 7 days following administration. On Day 8, dose administration was escalated to 500 times the proposed human dose level (132.15 µg/kg; 2643 µg/m²). Again, no adverse clinical observations were observed for the week following administration. On Day 15, 1 naïve male and female dog were administered 132.15 µg/kg P1 to confirm the NOAEL. Body weights and body temperatures were normal for all dogs for the duration of the study except for a minor body temperature decrease at the 24 hr time point for one dog administered 26.43 µg/kg P1 on Day 1. The minor change of body temperature for this dog is considered to be a sporadic variation and not test article-related.

There were no test article related CV effects, including ST segment abnormalities, pronounced U waves, nor T wave changes such as changes in polarity, increased amplitude, or flattening seen in any of the dogs after a single intravenous injection of 100x or 500x the human dose level of P1 for the duration of the study, except for dog #4, which was administered 132.15 µg/kg P1, had mild ST segment elevation on Day 15 at time points of 2 min and 4 hr. Also in dog #4, there was a change in T wave polarity from pre-dose positive T wave to 2 min negative T wave, to time 4 hr positive T wave, returning to negative T waves at time 24 hour. Dog #3 had increased QRS amplitude at baseline, pre-dose and throughout the study; and this was not due to a treatment effect.

The ST segment elevation changes seen in dog #4 administered 132.15 µg/kg P1 on Day 15 were transient, which may represent regional myocardial hypoxia and may have normalized at 24 hr post-dose. However, dog #3, also administered 132.15 µg/kg P1 on Day 15, had no adverse test article related CV effects. In addition, Dogs #1 and #2 were administered 132.15 µg/kg P1 on Day 8, with no test article related CV effects; therefore, the changes seen in dog #4 were not definitively due to a treatment related adverse effect.
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Several dogs (#s 1, 2 and 4) had left and/or right mean electrical axis (MEA) deviations at the baseline, pre-dose and post dose administration. These changes can be affected by many extra-cardiac factors and are not considered to be test article-related.

One dog (#2) had trivial hypotension at 4 hr post dose on Day 1. Animals with only reduced diastolic pressures were not considered to be hypotensive, as the diastolic pressure may be more likely to be erroneously low with oscillometric techniques; therefore, this effect is not considered to be test article related.

In conclusion, the study results confirmed that there were no overt cardiovascular or toxicity effects of P1 after a single intravenous (IV) injection of 100 times the proposed human dose level of P1 in Beagle dogs. Effects at 500 times the proposed human dose (132.15 µg/kg, 2643 µg/m²) are inconclusive, with minor, transient effects on ST segment elevation seen in a single dog out of 4 evaluated at this dose level. The NOAEL for P1 is considered to be greater than 26.43 µg/kg (528.6 µg/m²) and possibly less than 132.15 µg/kg (2643 µg/m²) via a single intravenous administration to male and female Beagle dogs. The maximum tolerated dose (MTD) was not determined, but is considered to be greater than 132.15 µg/kg (2643 µg/m²).
QUALITY ASSURANCE UNIT
Final Report and
Conflict of Interest Statement

SRI's Quality Assurance Unit assures that the study – Single Intravenous Administration Cardiovascular Safety Study of P1 in Male and Female Beagle Dogs, SRI Study No. M501-06 -- has been reviewed for adherence to U.S. Food and Drug Administration Good Laboratory Practice Regulations (21 CFR Part 58).

The following inspections were conducted during this study:

<table>
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<th>Date of Inspection</th>
<th>Date Findings Reported to Management/Study Director</th>
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<td>11-28-06</td>
<td>11-29-06</td>
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<td>Draft Final Report</td>
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<tr>
<td>Final Report Verification</td>
<td>04-12-07</td>
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This statement certifies that the personnel listed below participated in the inspections and audit of this study. These personnel have not been involved in the generation or evaluation of the data. Participation by the individuals listed below poses no conflict of interest.

Jeannette Robinson Pamela A. Pallakoff Esther Yau Thomas Bregante

[Signature]
Jeannette Robinson
SRI Quality Assurance Unit

4/12/07 Date
### Key Personnel

<table>
<thead>
<tr>
<th>Name</th>
<th>Title</th>
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<tr>
<td>Joseph Duran</td>
<td>Study Monitor</td>
</tr>
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<td>Jane Han, Ph.D.</td>
<td>Manager, Analytical Chemistry</td>
</tr>
<tr>
<td>Kristin MacDonald, D.V.M., D.A.C.V.I.M.</td>
<td>Veterinary Cardiologist</td>
</tr>
<tr>
<td>Hanna Ng, Ph.D.</td>
<td>Principal Investigator</td>
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<tr>
<td>Rhodora Ojerio</td>
<td>Veterinary Assistant</td>
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<td>Cecelia Paculba</td>
<td>Study Monitor</td>
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<td>Sandra Phillips</td>
<td>Director, Toxicology Support Services</td>
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<tr>
<td>Linda Rausch, M. S.</td>
<td>Study Director</td>
</tr>
<tr>
<td>Elaine Tran, B.S.</td>
<td>Supervisor, Dose Preparation</td>
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</table>
I. PURPOSE AND OBJECTIVE OF STUDY

The purpose of this study was to support an application for research or marketing purposes. This study, therefore, was performed in accordance with the U.S. FDA "Good Laboratory Practice for Nonclinical Laboratory Studies," as described in 21 CFR Part 58.

The objective of this study was to determine any potential cardiovascular or toxicity effects of P1 after a single intravenous injection of 10, 100, and 500 times the human dose level, and to determine a No Adverse Effect Level (NOAEL) in male and female Beagle dogs. Information from this study may be used to determine the suitability of the proposed human dose. The protocol and amendment are presented in Appendix A.

II. EXPERIMENTAL DESIGN

<table>
<thead>
<tr>
<th>Dose Level</th>
<th>Target Dose&lt;sup&gt;A&lt;/sup&gt;</th>
<th>µg/m²/day</th>
<th>µg/kg/day&lt;sup&gt;B&lt;/sup&gt;</th>
<th>Concentration (µg/mL)</th>
<th># Dogs Evaluated</th>
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<tbody>
<tr>
<td>10x Human Dose</td>
<td>52.86</td>
<td>2.643</td>
<td>1.06</td>
<td>1M/1F</td>
<td></td>
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<tr>
<td>100x Human Dose</td>
<td>528.6</td>
<td>26.43</td>
<td>10.6</td>
<td>1M/1F</td>
<td></td>
</tr>
<tr>
<td>500x Human Dose</td>
<td>2643</td>
<td>132.15</td>
<td>52.86</td>
<td>1M/1F</td>
<td></td>
</tr>
<tr>
<td>Confirm NOAEL</td>
<td>2643</td>
<td>132.15</td>
<td>52.86</td>
<td>1M/1F</td>
<td></td>
</tr>
</tbody>
</table>

<sup>A</sup>: Maximum human dose is 10 µg per 70 kg person, 10 µg/70 kg = 0.143 µg/kg x 37 (human surface area conversion) = 5.286 µg/m². Scaling for the dog gives 5.286 µg/m² / 20 (dog surface area conversion) = 0.2643 µg/kg as an equivalent human dose.

<sup>B</sup>: One male and one female dog were initially treated at a dose of 100x human dose. No effect was observed at 100x human dose, so the dose level was escalated to 500x the human dose in the same two animals (1/sex). No treatment at 10x human dose was performed. Two naive animals (1/sex) were used to confirm 500X human dose NOAEL.

Species and strain: Beagle dog

Route of administration and justification: Intravenous (iv), slow bolus. This is the route intended for human clinical trials; therefore iv injection was selected to model the intended route of human administration.

Frequency: Single dose for each escalation. First day of dose administration was Study Day 1.
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Data for each escalation was evaluated by the Study Director before determining the next dose to be administered.

Dose Administration
Volume: 2.5 ml/kg

III. MATERIALS AND METHODS

A. Test and Control Articles

1. Test Article: P1 (PBR28)

   Molecular Weight: 348.40
   Manufacturer: National Institute of Mental Health, NIH
   Supplier: National Institute of Mental Health, NIH
   Lot No.: 103006
   Physical Description: Off white powder
   Storage Conditions: 18° to 23°C

   Characterization of Test Article: Characterization, identity, and purity of the test article was the responsibility of the Sponsor. A Certificate of Analysis (C of A), with information of identity and purity, has been included in the final report. The C of A does not include information on stability of the test article; however, SRI performed chromatographic purity of the test article before study initiation and after study termination to cover the duration of the study. These results have been used for evaluation of the test article stability. The chromatographic purity was calculated by normalized peak area percentage. The same HPLC method was used in concentration and purity analyses of the dose formulations.

2. Control Article/Vehicle: 5% ethanol (v/v) and 95% (v/v) sterile saline

   Ethanol: Ethyl Alcohol 200 Proof, USP
   Manufacturer: Pharmco (Brookfield, CT)
   Lot No.: AC2057
   Physical Description: Clear, colorless liquid
   Storage Conditions: 18° to 28°C
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Saline: 0.9% Sodium Chloride for Injection, USP
Manufacturer: Baxter Healthcare (Deerfield, IL)
Supplier: VWR International, Inc. (Brisbane, CA)
Lot No.: C686022
Physical Description: Clear, colorless liquid
Storage Conditions: 20° to 23°C

Water: Sterile Water for Injection, USP
Manufacturer: Baxter Healthcare (Deerfield, IL)
Lot No.: C673293
Physical Description: Clear, colorless liquid
Storage Conditions: 18° to 28°C

Sodium Hydroxide: Sodium Hydroxide DiLut-IT® Analytical Concentrate, 0.1 N
Manufacturer: J.T. Baker (Phillipsburg, NJ)
Lot No.: B03426
Physical Description: Clear, colorless liquid
Storage Conditions: used immediately upon receipt

Characterization of Control Article/Vehicle: Information on the identity, purity, and stability was obtained by recording all of the pertinent information provided on the container labels or from the Certificate of Analysis (C of A).

Preparation of Dose Formulations: Dose solutions for all three escalation formulations were prepared by dissolving the appropriate amount of test article in ethanol, mixed for 30 min, sonicated for 30 sec, diluted with saline to achieve the target concentration, and mixed 22 min (including monitoring of pH). The initial pH of the solution was 5.58. The pH was adjusted using 0.1N NaOH DILUT_IT® (Lot B03426; Phillipsburg, NJ). The final pH was 6.76. The solution was mixed for another 15 minutes using a stir bar. Solutions were sterile filtered into sterile serum vials. Dose formulations were stored refrigerated at 3.0° to 4.1°C until use.
3. **Characterization of Dose Formulations:**

   Determination of formulation stability and homogeneity was conducted by SRI prior to, and concurrently with, the study (see Attachment A). Dose formulations were used within the window of stability determined in the stability study. Results of the dose verification have been included in Appendix B.

   Dose formulation concentrations were determined using an analytical method developed for these compounds and described in Attachment A. The analytical methods have been provided in Appendix B.

4. **Test Article Handling:**

   At the minimum, the test article and dose formulations were handled with the use of eye protection, latex gloves, and a protective smock or laboratory coat.

   An MSDS was provided which included recommended procedures for safe handling of the test article, for handling an accidental spill, and for disposal of the waste contaminated with the test articles.

5. **Disposition:**

   Unused bulk test article will be returned to the Supplier following the end of the study. Residual dose solutions will be discarded upon acceptance of the final report unless otherwise instructed by the Sponsor.

6. **Method for Assuring Correct Dose:**

   The test article was weighed with calibrated balances. The preparation and administration of each formulation was properly documented and records have been maintained showing the amount administered to each animal.

**B. Test System**

**Species:** Dog  
**Strain:** Beagle
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<table>
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<tr>
<th><strong>Supplier:</strong></th>
<th>Marshall Farms (North Rose, New York)</th>
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<tr>
<td><strong>Gender:</strong></td>
<td>Male and female</td>
</tr>
<tr>
<td><strong>Age at Initiation:</strong></td>
<td>15 months</td>
</tr>
<tr>
<td><strong>Weight at Initiation:</strong></td>
<td>8.3 to 13 kg</td>
</tr>
<tr>
<td><strong>Number of Animals:</strong></td>
<td>4 assigned to test</td>
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**Animal Care:** General procedures for animal care and housing was in accordance with the National Research Council (NRC) *Guide for the Care and Use of Laboratory Animals* (1996) and the Animal Welfare Standards incorporated in 9 CFR Part 3, 1991.

**Quarantine:** These dogs were used previously for another study and went through at least 3 weeks of wash out period. The dogs appeared normal before the initiation of the study. Baseline and pre-dose information on cardiovascular evaluation parameters and blood pressure were obtained.

**Housing:** Housed 1-2 dogs in a 4' x 8' wire mesh enclosure (run) with a concrete floor.

**Light Cycle:** 12 hr light/12 hr dark

**Temperature:** 60° to 75°F; protocol states 64° to 84°F. Unable to determine duration since digital hygrothermograph was used but minimum adverse affects to the study are expected due to this deviation. The temperature was not recorded on Day 5 due to a malfunction of the instrument. This is not expected to have adversely affected the study since the animals appeared healthy during this time period.

**Humidity:** 25-88%, this deviated from the protocol-specified range of 30-70%; however, this is not expected to affect the study since the animals appeared healthy. The humidity was not recorded on Day 5 due to a malfunction of the instrument. This is not expected to have adversely affected the study since the animals appeared healthy during this time period.
Ventilation: At least 10 room volumes per hour, with no recirculation of air.

Food: Harlan Teklad 2025-C Certified Canine Chow (Hayward, CA). Dogs were given their daily ration of food. The quantity of the daily ration was sufficient to meet nutritional requirements. Feed is analyzed periodically to ensure that contaminants known to be capable of interfering with the study and reasonably expected to be present in such feed are not present at levels that would impact the study. Documentation of feed analyses is maintained in the study records.

Water: Public water supply was provided *ad libitum*. No contaminants that could interfere with and affect the results of the study are expected to have been present in the water, based on previous analysis reports. Copies of annual analysis reports will be maintained at SRI for reference.

Identification: Individually identified by a unique ear tattoo.

Test System Justification: This is an accepted species to support studies of compounds used or intended for use in humans.

Welfare of the Animals: Every effort was made to minimize, if not eliminate, pain and suffering in all animals in this study.

Assignment of Animals to Study:


Group Assignment: Manually assigned to treatment groups.

C. Experimental Procedure (In-Life Evaluations)

Mortality/Morbidity: At least once daily.

Clinical Observation: Observations were made immediately post dose and daily for the remainder of the study.

Body Weights: Prior to dosing on each escalation.
(Days 1 and 8).

**Cardiovascular Evaluation**

**Parameters Evaluated:** Electrocardiograms (ECG), heart rate, respiratory rate, and blood pressure (indirect) recordings from Prestudy, Day 8, and Day 15 were evaluated by Dr. Kristin MacDonald, D.V.M., D.A.C.V.I.M. The cardiologist’s report is included in Appendix E-1.

**Frequency:** All cardiac measurements were performed pre-dose and initiated at 2, 30, 60, 120, and 240 min post-dose. ECGs were recorded continuously between 2 and 15 min post dose. In addition, measurements were collected at 24 hr post-dose.

**Method:** All ECGs were measured using a minimum of 4 leads and a lead II rhythm strip recorded at 25 mm/sec paper speed at standard sensitivity (1mm/mV). Blood pressure was measured using a SurgiVet V6004 oscillometric monitor.

ECGs were evaluated for HR, rhythm, and mean electrical axis (MEA). HR and MEA were obtained from the automatic calculations generated by the electrocardiograph, when available. The rhythm was determined subjectively. P-wave duration, PR interval, QRS duration, and QT interval were calculated from manually recorded measurements. Each parameter was averaged from three measurements from three different beats. The corrected QT interval (QTc) was calculated using Fredericia’s formula.

RR was measured by counting the number of inhaled breaths in a 15-second interval and multiplying by 4 to determine the number of respirations per minute.

BP was measured five times, when possible, at each time point using an oscillometric monitor, according to the manufacturer’s instructions, with the cuff placed on the right or left forelimb. Average BPs were calculated from measurements recorded at each time point.
Body Temperature:

Frequency: Body temperature measurements were performed pre-dose and initiated at 2, 30, 60, 120, and 240 min post-dose.

Method: Body temperature was obtained by a digital rectal thermometer.

D. Evaluation of Data:

Statistical Test: No statistical evaluations were performed.

E. Control of Bias

When evaluating the animals, the technical staff was aware of the each animal’s treatment history. However, based on the relatively objective endpoints to be examined, bias is not expected to have influenced the results of the study.

F. Good Laboratory Practices Compliance

This study is intended to be submitted to and reviewed by the U.S. Food and Drug Administration (FDA) or an equivalent regulatory agency and, therefore, was performed in accordance with the U.S. FDA "Good Laboratory Practice for Nonclinical Laboratory Studies," as described in 21 CFR Part 58, with the following exceptions.

- Animal water and food analysis were not performed under GLP compliance by the vendors.

- A digital temperature station, manufactured by La Crosse Technology, Inc, was used to measure the temperature and humidity of the animals’ housing conditions during the course of the study. This instrument measures minimum and maximum readings for the day and does not provide continuous measurement of the temperature and humidity.

- Characterization of the test article was the responsibility of the Sponsor. The Sponsor submitted a Certificate of Analysis (C of A) that included information on chemical identity and purity. The C of A of the test article was not conducted in accordance with the U.S. FDA GLP compliance. The C of A did not include information on stability of the test article; however, SRI performed chromatographic purity of the test article by measuring both before and after several days of test article in the formulation. Purity was calculated by normalized peak area percentage and purity results were used for evaluation of the test article stability.
G. Retention of Records and Study Samples

The original protocol and amendments, original final report, and all raw data, supporting documents, and records specific to this study will be retained and stored in the Records Center at SRI International, 333 Ravenswood Avenue, Menlo Park, CA 94025. All records will be maintained for a period of at least 5 years. At the end of the retention period, the Sponsor will be contacted regarding further disposition of these records.

IV. RESULTS AND DISCUSSION

A. Mortality/Morbidity and Clinical Observations

Individual animal clinical observations are presented in Appendix C.

All animals survived the duration of the study. One female administered 26.43 µg/kg P1 had slightly loose stool on Day 8. No adverse clinical observations were seen for any of the other dogs at any dose level for the duration of the study.

B. Body Weights

Individual animal body weights are presented in Appendix D.

All animals consistently gained body weight for the duration of the study compared with their respective Day 1 body weights.

C. Cardiology

The cardiologist’s report, and individual animal blood pressure and ECG data are presented in Appendix E.

Electrocardiograms (ECG)

There were no rhythm abnormalities at any time point in the study.

Dog #3, administered 132.15 µg/kg P1, had persistently increased QRS amplitude at baseline and at Day 15. Dog #4, administered 132.15 µg/kg P1 had mild ST segment elevation on Day 15 at 2 min and 4 hr post dose administration. Also for dog #4 at the baseline pre-study ECG, the T waves were negative; however, there was a change in T wave polarity from positive at pre-dose, to negative T wave at 2 min post dose, positive T wave at 4 hr, returning to negative at 24 hr post dose administration.

At baseline, dogs #1 and #2 had left axis deviations, and dog #4 had a significant left axis deviation. On Day 1, Dog #1, administered 26.43 µg/kg P1, had right axis
deviation at pre-dose, 2 min, and 2 hr post dose administration, and left axis deviation at 30 min, 1, and 24 hr post dose administration. Dog #2, administered 26.43 µg/kg P1, had left axis deviation at pre-dose, and significant left axis deviations at 30 min, 1, 2, 4, and 24 hr post dose administration. On Day 8, dog #1, administered 132.15 µg/kg P1, had trivial left axis deviations at 30 min, 2, and 4 hr post dose administration. Dog #2, also administered 132.15 µg/kg P1, had significant left axis deviations at pre-dose, 30 min, 2, and 24 hr post dose administration, and left axis deviations at 2 min, 1, and 4 hr post dose administration. On Day 15, dog #4 had significant left axis deviations at pre-dose, 30 min, 2 hr post dose, and significant right axis deviations at 2 min, 4, and 24 hr post dose, as well as a trivial right axis deviation at 1 hr post dose administration of 132.15 µg/kg P1. In animals with mean electrical axis (MEA) deviations, there were normal QRS durations, indicating there were no partial bundle branch blocks. The axis deviations do not reflect persistent, significant conduction system disturbances and are likely affected by subtle changes in body position or electrode placement. MEA can be affected by many extra-cardiac factors including thoracic shape, body position, inappropriate or incorrect lead placement. MEA is a poor indication of left or right ventricular chamber size, and is mainly useful to determine presence of severe right ventricular hypertrophy or to evaluate for intraventricular conduction defects such as bundle branch block. Dog #4 had wide fluctuations of the MEA at Day 15, which may represent positional changes of the body or may be due to altered intraventricular conduction, since there was also ST segment and T wave changes at the same time points.

**Blood Pressure**

On Day 1, dog #2, administered 26.43 µg/kg P1, had a trivial decrease in diastolic blood pressure (55 mmHg) 2 hr post dose administration, and a trivial hypotension with reduced diastolic, systolic, and mean arterial pressure (55 mmHg, 95 mmHg, and 73 mmHg respectively) 4 hr after dose administration. On Day 8, dog #2, administered 132.15 µg/kg P1, had decreased diastolic pressure (48 mmHg) 24 hr post dose administration. On Day 15 no abnormalities in blood pressure were seen.

**Body Temperature**

Body temperatures were normal for all dogs for the duration of the study except for dog #2 administered 26.43 µg/kg P1 on Day 1 which had a slightly decreased body temperature of 99.7°F at 24 hr post dose administration compared with pre-dose body temperature of 101.5°F. The minor change of body temperature for this dog is considered to be a sporadic variation and not test article-related.

V. **CONCLUSIONS**

This study involved three (Days 1, 8, and 15) dose administrations of P1 separated by one week intervals. Body weights were collected to determine dose calculations for each dose administration. Cardiovascular (CV) baseline data was collected prior to each dose
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administration including electrocardiogram (ECG), heart rate, respiratory rate, and blood pressure (indirect). Body temperature was also collected. Following administration of P1, each CV parameter was collected at 2, 30, 60, 120, 240 min, and 24 hr post dose administration. Clinical observations were made immediately post dose and daily thereafter for the duration of the study.

The first IV administration of P1 was 26.43 µg/kg (528.6 µg/m²), 100 times the proposed human dose level. No adverse signs were observed for 7 days following administration. On Day 8, dose administration was escalated to 500 times the proposed human dose level (132.15 µg/kg; 2643 µg/m²). Again, no adverse clinical observations were observed for the week following administration. On Day 15, 1 naïve male and female dog were administered 132.15 µg/kg P1 to confirm the NOAEL. Body weights and body temperatures were normal for all dogs for the duration of the study except for a minor body temperature decrease 24 hr post dose for one dog administered 26.43 µg/kg P1 on Day 1. This minor change of body temperature is considered to be a sporadic variation and not test article related.

There were no test article related CV effects, including ST segment abnormalities, pronounced U waves, nor T wave changes such as changes in polarity, increased amplitude, or flattening seen in any of the dogs after a single intravenous injection of 100x or 500x the human dose level of P1 for the duration of the study, except for dog #4, which was administered 132.15 µg/kg P1, had mild ST segment elevation on Day 15 at time points of 2 min and 4 hr. Also in dog #4, there was a change in T wave polarity from pre-dose positive T wave to 2 min negative T wave, to 4 hr positive T wave, returning to negative T waves at 24 hr.

The ST segment elevation changes seen in Dog #4 administered 132.15 µg/kg P1 on Day 15 were transient, which may represent regional myocardial hypoxia and may have normalized at 24 hr post-dose. However, dog #3, also administered 132.15 µg/kg P1 on Day 15, had no adverse test article related CV effects. In addition, Dog 1 and 2 were administered 132.15 µg/kg P1 on Day 8, with no test article related CV effects. Due to the small number of dogs tested (2 males and 2 females), it is not clear whether the ST segment and T wave changes seen in Dog #4 are conclusively linked to a test article related effect; however, 3 of the 4 dogs tested in this study at this dose level had no adverse signs related to ST segment and T wave changes. In addition, the CV changes were transient, only seen at 2 min and 4 hr post dose administration, and returned to normal by 24 hr. The significance of the ST segment elevation related to test article-treatment in dog #4 may be minimal, but not conclusive.

Several dogs (#s 1, 2 and 4) had left and/or right mean electrical axis (MEA) deviations at the baseline, pre-dose and post dose administration. These changes can be affected by many extra-cardiac factors and are not considered to be test article-related.

There was no treatment effect on blood pressure observed that was considered to be related to test article treatment.

In conclusion, the study results confirm that there are no overt cardiovascular or toxicity effects of P1 after a single intravenous (IV) injection of 100 times the proposed human dose
level of P1 in Beagle dogs. Effects at 500 times the proposed human dose (132.15 µg/kg, 2643 µg/m²) are inconclusive, with minor, transient effects on ST segment elevation seen in a single dog out of 4 evaluated at this dose level. The NOAEL for P1 is considered to be greater than 26.43 µg/kg (528.6 µg/m²) and possibly less than 132.15 µg/kg (2643 µg/m²) via a single intravenous administration to male and female Beagle dogs. The maximum tolerated dose (MTD) was not determined, but is considered to be greater than 132.15 µg/kg (2643 µg/m²).