VALIDATION OF THE USE OF A TELEMETRY STUDY TO MEASURE HEMODYNAMIC, ELECTROCARDIOGRAPHIC AND RESPIRATORY PARAMETERS IN THE CONSCIOUS DOG

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Abstract
Animal research remains a critical component to safety evaluation of candidate drugs, with telemetry providing insightful cardiorespiratory data. Safety pharmacology supports reducing animal numbers while optimizing study outcomes. Therefore, Beagle dogs were instrumented with Data Sciences International (DSI) implants (L11/L11R), and a structured Latin Square crossover design was used to ascertain the discrete effects of verapamil, dofetilide and doxapram. Hemodynamic, electrocardiographic and body temperature data were captured using the DSI Ponemah v5 data capture telemetry system. EMKA ECG Auto v3 was used to interrogate the ECG signals. Baseline values for all groups were as expected for the telemetered Beagle dog. Verapamil at 15 mg/kg significantly decreased blood pressure and caused a substantive increase in the PR interval over most of the study duration, with 2° atrioventricular block developing in four of eight dogs. Dofetilide at 0.3 mg/kg significantly increased the PR interval during the 6-hour monitoring period, while 0.1 mg/kg and 0.3 mg/kg dofetilide significantly increased the corrected QTcH interval. Increases in both delayed and lost 1:1 atrioventricular conduction were noted in all dogs following 0.3 mg/kg dofetilide, while supraventricular ectopy increased in a dose-dependent fashion. Administration of 4 mg/kg doxapram elicited significant increases in blood pressures over the 4-hour monitoring period as well as a notable increase in respiratory rate, tidal volume and minute volume shortly after administration. Thus, the expected effects of each test compound were accurately detected in Beagle dogs, validating the usage of an optimized safety pharmacology study to meet cardiorespiratory ICH guidelines.

Introduction
Three pharmacological validation studies were conducted on male Beagle dogs instrumented with DSI telemetry implants to assess the ability to detect the expected effects of verapamil, dofetilide and doxapram. Verapamil, an L-type calcium channel agonist, was used to assess the ability to detect effects on hemodynamic parameters. Dofetilide, a potassium channel agonist, was used to assess the ability to detect effects on ECG parameters. Doxapram, a stimulant of the central and peripheral chemoreceptors that produces an increase in respiratory minute volume, HR and blood pressure was used was used to assess the ability to detect effects on respiratory parameters as well as standard cardiovascular endpoints.

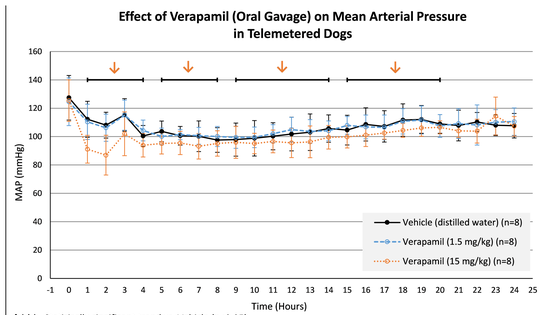
Methods
Experimental Plan:

- A total of 24 Beagle dogs (16 M/8 F) were instrumented with either DSI L11 or L11R implants. Animal weights ranged from 7.9 – 11.9 kg (M) and 6.6 – 10.6 kg (F) prior to study initiation. Animals were dosed with vehicle, verapamil (4M/4F), dofetilide (4M/4F) or doxapram (8M) via oral gavage on each dosing day following a structured Latin Square design, with a minimum of 2 washout days between dose administrations.
- Body weights were obtained during the acclimation period and then at protocol-defined time points during each study. Clinical observations were conducted at least twice daily.

Data Analysis
Hemodynamic, ECG and BT data were captured using the DSI Ponemah v5 data capture telemetry system. EMKA ECG Auto v3 was used to interrogate the ECG signals. These recordings were obtained from all dogs from approximately 1 hour prior to dosing to a protocol-defined time point post dosing for each test compound (verapamil: 24 hours; dofetilide: 6 hours; doxapram: 4 hours). Mean arterial pressure (MAP), systolic arterial pressure (SAP), diastolic arterial pressure (DAP), heart rate (HR) and pulse pressure (PP) were ascertained via the femoral artery telemetry catheter. PR interval, QRS duration and QT/QTc interval were obtained via the telemetered ECG waveforms via an intravascular approach with electrode placement in the cranial vena cava. QT interval was corrected for heart rate changes by the individualized Holzgrefe correction method. BT was determined via the temperature sensor located within the telemetry implant.

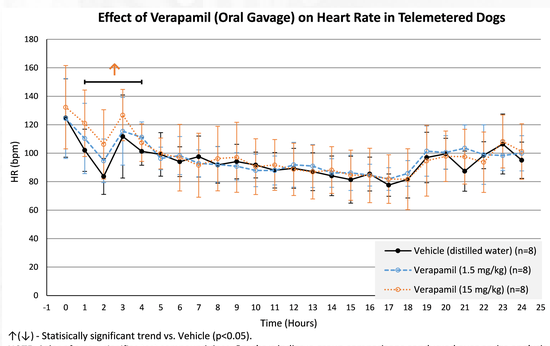
Statistical analyses were conducted using SAS® software, with statistical significance reached at the .05 level. Baseline-corrected, vehicle-subtracted (double delta) comparisons were used as the primary analysis method for test compound data to further assess any statistically significant effects.

Results
VERAPAMIL (1.5 mg/kg and 15 mg/kg, dosed via oral gavage)
- Baseline hemodynamic and ECG values for all groups, including those obtained after vehicle dosing (distilled water), were within expected ranges for the telemetered Beagle dog



↑(↓) - Statisically significant trend vs. Vehicle (p<0.05).
NOTE: Color of arrow signifies test compound dose. Brackets indicate group comparisons conducted over entire analysis phase.

Figure 1. Mean Arterial Pressure (MAP) values were significantly decreased following administration of 15 mg/kg verapamil whereas there was no effect on blood pressure following administration of either vehicle or 1.5 mg/kg verapamil.



↑(↓) - Statisically significant trend vs. Vehicle (p<0.05).
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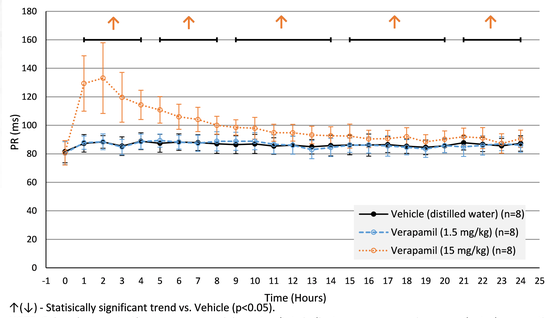
Figure 2. Heart rate (HR) was significantly increased from 0–4 hours post dose following administration of 15 mg/kg verapamil. No notable effect was observed on HR following vehicle or 1.5 mg/kg verapamil.



↑(↓) - Statisically significant trend vs. Vehicle (p<0.05).
NOTE: Color of arrow signifies test compound dose. Brackets indicate group comparisons conducted over entire

Figure 3. A statistically significant increase in the PR interval was observed for the duration of the post-dose monitoring period following administration of 15 mg/kg verapamil. No notable effect was observed on the PR interval following vehicle or 1.5 mg/kg verapamil.

Hemodynamic Findings:

- No gender differences were noted across hemodynamic parameters following either dose of verapamil
- No statistically significant effects were noted on any hemodynamic parameter following administration of 1.5 mg/kg verapamil
- A significant decrease in MAP, SAP and DAP was observed following administration of 15 mg/kg verapamil from 1–20 hours post dose (Figure 1) while a significant increase in HR was noted from 1–4 hours post dose following administration of 15 mg/kg verapamil (Figure 2)
- A significant decrease in pulse pressure was noted from 9–20 hours following administration of 15 mg/kg verapamil

Electrocardiographic Findings:

- No statistically significant effects were observed on the QRS duration following either dose of verapamil.
- A statistically significant increase in the PR interval was observed following administration of 15 mg/kg verapamil from 1–24 hours post dose (Figure 3)
- Gender differences were noted in the QT and QTcH intervals
- Significant increases in the QTcH interval were noted in female Beagle dogs from 15–24 hours post dose whereas no significant effects on QTcH were noted in male Beagle dogs
- Second degree atrioventricular (AV) block, manifest as a loss of 1:1 AV conduction (non-conducted P waves), was observed in 4 animals (3 males and 1 female) following administration of 15 mg/kg verapamil only. This dysrhythmia started approximately 20 minutes post dose and resolved to normal sinus rhythm (NSR) by approximately 2–3 hours post dose in 3 of the 4 animals.

Body Temperature Findings:

- Baseline BT for vehicle and both verapamil doses (1.5 mg/kg and 15 mg/kg) was 37.5 ± 0.5 °C.
- No significant changes in BT were observed following verapamil at either dose.

DOFETILIDE (0.1 mg/kg and 0.3 mg/kg, dosed via oral gavage)
- Baseline hemodynamic and ECG values for all groups, including those obtained after vehicle dosing (0.4% methylcellulose in distilled water), were within expected ranges for the telemetered Beagle dog
- No gender differences were noted in hemodynamic or ECG parameters following vehicle or dofetilide administration

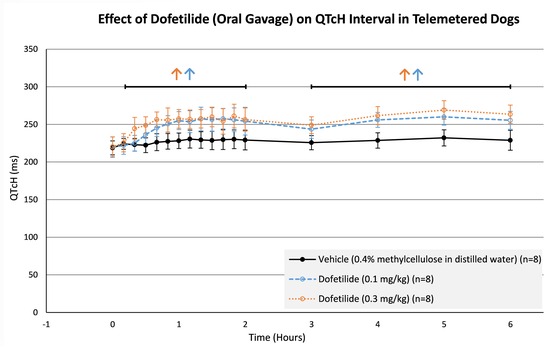


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Figure 4. A statistically significant increase in the corrected QT interval (QTcH) was observed following administration of both 0.1 mg/kg and 0.3 mg/kg dofetilide.

Summary of Arrythmias and Abnormal Beats for Vehicle and Dofetilide Doses (0.1 mg/kg and 0.3 mg/kg)			
Finding	Number of Occurrences		
	Vehicle 0 mg/kg (n=8)	Dofetilide 0.1 mg/kg (n=8)	Dofetilide 0.3 mg/kg (n=8)
Sum of AV Block 1st	58	173	3289
Sum of AV Block 2nd	114	61	1433
Sum of V Ectopic	2	0	0
Sum of Junctional	3	2	57
Sum of V Ectopic Single	2	0	0
Sum of SVE_Abr	2	153	6999
Sum of PVC Single	2	0	0
Sum of Sinus Pause	0	0	1
Sum of V Bigeminy	0	0	0
Sum of V Couplet	0	0	0
Sum of V Couplet Series	0	0	0
Sum of V Escape Single	0	0	0
Sum of V Interpolated	0	0	0
Sum of V Run	0	0	0
Sum of V Trigeminy	0	0	0
Sum of V Triplet	0	0	0
Sum of SVE_Abr_Couplet	0	13	975
Sum of SVE_Abr_Run	0	0	471
Sum of SVE_Abr_Triplet	0	0	3
Sum of SVE_Abr_Bigeminy	0	2	57
abr, aberrant; AV, atrioventricular; SVE, Supraventricular ectopy; V, ventricular			

Table 1. Summary of Arrhythmias and Abnormal Beats for Vehicle and Dofetilide Doses (0.1 mg/kg and 0.3 mg/kg)

Hemodynamic Findings:

- There were no statistically significant changes in MAP, SAP, DAP, HR or PP following administration of dofetilide at either 0.1 mg/kg or 0.3 mg/kg

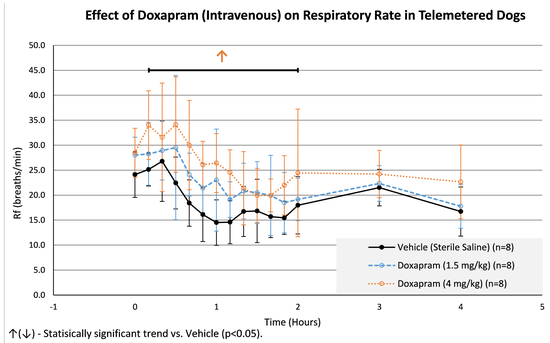
Electrocardiographic Findings:

- No significant effects occurred for QRS duration following either dose of dofetilide
- PR interval was significantly increased following 0.1 mg/kg dofetilide from 10–120 minutes post dose and following 0.3 mg/kg dofetilide from 10 minutes to 6 hours post dose
- QT/QTcH intervals were stable during the 6-hour monitoring period following administration of vehicle while both QT/QTcH intervals were increased significantly from 10 minutes to 6 hours post dose following both dofetilide doses (Figure 4)
- Arrythmias and abnormal beats were assessed by DSI Data Insights software (Table 1)
- There was almost zero incidence of arrhythmias in the vehicle treated dogs except for transient atrioventricular block, a normal variant in the Beagle dog.
- Increases in both delayed and lost 1:1 AV conduction (Sums of AV Block 1st and 2nd) were noted at 0.3 mg/kg dofetilide, while supraventricular ectopy (Sum of SVE_abr) increased in a dose-dependent fashion.
- Male dogs appeared to have higher incidence of SVE than females after 0.3 mg/kg dofetilide.

Body Temperature Findings:

- Baseline BT values were comparable for all groups with vehicle and dofetilide at 0.1 mg/kg and 0.3 mg/kg: 38.0 ± 0.4 °C, 38.1 ± 0.3 °C and 38.1 ± 0.4 °C, respectively
- No substantive effects on BT were observed following administration of either 0.1 mg/kg or 0.3 mg/kg dofetilidePage Break

DOXAPRAM (1.5 mg/kg and 4 mg/kg, dosed via oral gavage)
Baseline hemodynamic and ECG values for all groups, including those obtained after vehicle dosing (0.9% saline for injection, USP), were within expected ranges for the telemetered Beagle dog



↑(↓) - Statisically significant trend vs. Vehicle (p<0.05).
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Figure 5. Respiratory rate was significantly increased following administration of 4 mg/kg doxapram.

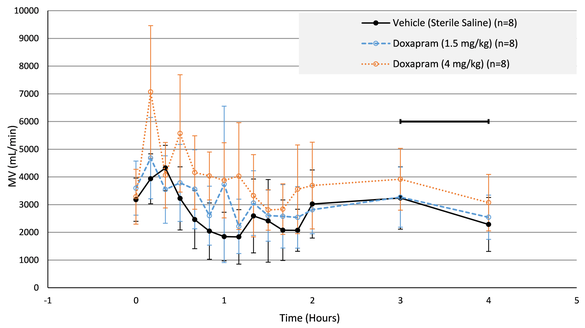

Figure 6. Minute volume was significantly increased for the majority of the post dose monitoring period following administration of 4 mg/kg doxapram while a singular time point reached statistical significance following administration of 1.5 mg/kg doxapram.

Hemodynamic Findings:

- Arterial pressures (MAP, SAP and DAP) and pulse pressure were significantly elevated following 1.5 mg/kg doxapram for the majority of time points from 10–70 minutes post dose while arterial pressures and PP were significantly elevated following 4 mg/kg doxapram for the majority of the 4-hour post dose monitoring period
- Heart rate was significantly increased following 4 mg/kg doxapram for the majority of the 4-hour monitoring period

Electrocardiographic Findings:

- PR interval and QRS duration were stable following administration of vehicle and either 1.5 mg/kg or 4 mg/kg doxapram
- QT/QTcH interval was stable following administration of vehicle and both doses of doxapram

Respiratory Findings:

- Respiratory rate was significantly increased from 10–120 minutes post dose following administration of 4 mg/kg doxapram while a modest increase was also noted during this time following 1.5 mg/kg doxapram, the increase did not meet statistical significance (Figure 5)
- Tidal volume was significantly increased at sporadic time points following administration of 4 mg/kg doxapram (10, 50, 70 and 110 minutes post dose)
- Minute volume was significantly increased for the majority of the experimental duration following 4 mg/kg doxapram (Figure 6)

Body Temperature Findings:

- BT was significantly increased from 10–120 minutes post dose following administration of 4 mg/kg doxapram; however, BT effects ranged from -1 to +2 percentage points (delta-delta) during this period. As such, effects are considered inconsequential.
- No other significant effects on BT were noted during the study.

Conclusions
Each validation study showed the ability to detect the expected effects on respiratory and/or hemodynamic and electrocardiographic end points.

- Verapamil elicited a significant decrease in blood pressure and a significant increase in PR interval following the high dose (15 mg/kg)
- Dofetilide led to a significant increase in the QTcH interval at both 0.1 mg/kg and 0.3 mg/kg, with further analysis detecting arrhythmias (both delayed and lost 1:1 AV conduction at 0.3 mg/kg dofetilide and supraventricular ectopy at 0.1 mg/kg and 0.3 mg/kg dofetilide)
- Doxapram significantly increased respiratory rate, minute volume, HR and blood pressure following the 4 mg/kg dose