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Characterization of an investigative safety pharmacology model to assess comprehensive cardiac function and structure in chronically instrumented conscious beagle dogs

Christopher P. Regan *, Gary L. Stump, Theodore J. Detwiler, Li Chen, Hillary K. Regan, David B. Gilberto, Joseph J. DeGeorge, Frederick J. Sannajust

Department of Safety and Exploratory Pharmacology, Merck Research Laboratories, West Point, PA 19486, USA

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ABSTRACT

Introduction: There has been an increasing need to conduct investigative safety pharmacology studies to complement regulatory-required studies, particularly as it applies to a comprehensive assessment of cardiovascular (CV) risk.
Methods: We describe refined methodology using a combination of telemetry and direct signal acquisition to record concomitant peripheral hemodynamics, ECG, and left ventricular (LV) structure (LV chamber size and LV wall thickness) and function, including LV pressure-volume (PV) loops to determine load independent measures of contractility (end systolic elastance, Ees, and preload recruitable stroke work, PRSW) in conscious beagle dogs. Following baseline characterization, 28 days of chronic rapid ventricular pacing (RVP) was performed and cardiac function monitored: both as a way to compare measures during development of dysfunction and to characterize feasibility of a model to assess CV safety in animals with underlying cardiac dysfunction.
Results: While ±dP/dT decreased within a few days of RVP and remained stable, more comprehensive cardiac function measurements, including Ees and PRSW, provided a more sensitive assessment confirming the value of such endpoints for a more clear functional assessment. After 28 days of RVP, the inodilator pimobendan was administered to further demonstrate the ability to detect changes in cardiac function. Expectedly pimobendan caused a leftward shift in the PV loop, improved ejection fraction (EF) and significantly improved Ees and PRSW.
Discussion: In summary, the data show the feasibility and importance in measuring enhanced cardiac functional parameters in conscious normal beagle dogs and further describe a relatively stable cardiac dysfunction model that could be used as an investigative safety pharmacology risk assessment tool.

1. Introduction

The foundation of Safety Pharmacology (SP) is based strongly in regulatory required studies; however, SP continues to evolve toward a balance of regulatory and investigative science. This continued evolution is necessary to determine mechanism of action, to drive safety-related decision earlier in drug development, and to enable more comprehensive risk assessment throughout all phases of drug development. To accomplish this, expanded cardiovascular in vivo assessment toward "second tier" physiologic endpoints, for example, cardiac function can be warranted. In fact, several recent studies have sought to expand SP investigation of hemodynamics through a better understanding of indices of contractility in normal dogs and non-human primate (Markert et al., 2007, 2012; Cools et al., 2014; Guth et al., 2015). While the second tier endpoints are not required under ICH guidelines, ICH S7A describes the utility of studies including such endpoints in follow up studies (Food and Drug Administration, 2001) and the scientific utility of such work has been previously described (Bass et al., 2008; Cavero, 2009; Taylor et al., 2007). Moreover, determination of test agent-dependent effects in animals with underlying cardiovascular dysfunction (ex: disease models), is of continued interest. Use of such models in SP remains a major challenge and there is no formal guidance defining their use; however, there are cases where such models may be warranted to better understand risk liability or mechanism of action in addition to assessment in normal animals. More to the point, "second tier" endpoints alone and their use in combination with disease models can serve a critical complementary role to better inform on potential risk profile and/or determine mechanism of action of potential off target effects. This can help guide decision making in not only discovery and preclinical development, but clinical development as well.

In addition to peripheral hemodynamics, direct test agent-dependent effects on cardiac function can be of critical importance to the complete cardiovascular safety profile. There are many options to gain additional insight to cardiac function, from direct pressure

* Corresponding author at: Merck Research Laboratories, Department of Safety Pharmacology, 770 Sumneytown Pike WP45A-B226, West Point, PA 197486, USA.

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measurements to imaging techniques (Norton et al., 2009; Markert et al., 2007, 2012; Cools et al., 2014; Hockings et al., 2003). Among them there has been significant work within SP toward characterization and utilization of dP/dT, the first derivative of left ventricular (LV) pressure waveform, as an index of cardiac contractility (Guth et al., 2015; Markert et al., 2012; Cools et al., 2014). However, it is well-accepted that the gold standard to assess cardiac function is the pressure-volume (PV) loop, (Suga et al., 1973; Sagawa, 1981; Kass et al., 1987), in part because direct function, independent of loading conditions, can be obtained. PV loops are most readily determined via one of two methods: implanted sonomicrometer crystals, technology originally developed in the mid-1950s (Rushmer et al., 1956) or via the use of conductance catheters (Kass et al., 1986). Conductance catheters are used currently more often than crystals for such data likely because they are more readily available, easier to instrument, and amenable to small and large animal studies. However, current conductance catheter technology is effectively limited to use in anesthetized animals and variability can be introduced by positional changes in the catheter: either within measurement sessions or across measurements. While significantly more information about hemodynamics and cardiac function can be derived from basal PV loops vs blood pressure and/or LV pressures alone, key intrinsic properties of the ventricle, specifically intrinsic contractility independent of loading conditions, require the addition of varying cardiac preload, most commonly accomplished by transient inferior vena cava occlusion (IVCo) (Crottogini, Willshaw, Barra, & Pichel, 1994; Sodums et al., 1984; Little et al., 1989).

To investigate the feasibility of conscious PV loop assessment in beagle dogs, we leveraged sonomicrometer technology and implantable pressure recording technology, along with techniques to enable transient IVC occlusion. We further instrumented animals with cardiac pacing electrodes to create cardiac dysfunction via chronic rapid ventricular pacing (RVP). Through this, we describe a model that can be utilized to further understand test agent-dependent effects on cardiac function in conscious beagle dogs and provide characterization of pacing-induced cardiac dysfunction in beagle dogs.

2. Methods

2.1. Statement on use and care of animals

All animal studies were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Research, Division on Earth and Life Studies, & National Research Council, 2011) and were approved by the Institutional Animal Care and Use Committee at Merck Research Laboratories, West Point.

2.2. Surgical instrumentation

Five adult, male beagle dogs (10–13 kg, 12–18 months of age, Marshall Biosciences) were anesthetized with propofol (6 mg/kg iv, to effect) followed by intubation and general anesthesia with isoflurane (1–3 vol% in oxygen, to effect). Using sterile surgical technique, a left thoracotomy was performed at the fifth intercostal space, and the pericardium was incised to expose the heart. Aortic and LV pressures and ECG were recorded via an ITS T27-G series implant (Konigsberg, Pasadena, CA). A pressure transducer was inserted through the LV apex and advanced into the LV cavity to record LV pressures. The descending aorta was then isolated and a section clamped. An incision was made in the thoracic aorta and the pressure probe inserted. The probe was secured with appropriate sutures and/or tissue glue. Wires exited the chest through a single location and the battery and electronics of the implant were placed in an intramuscular pocket on the left flank of the animal. A positive ECG lead was placed subcutaneously in an area over the LV with the aortic pressure transducer serving as the reference electrode to record Lead II ECG. To record LV dimensions and LV free wall thickness piezoelectric ultrasonic dimension crystals (Sonometrics Corporation, London Ontario, Canada) were implanted on the endocardial and epicardial surfaces to measure LV short axis LV free wall thickness. Briefly, two crystals (anterior-posterior, located one-half to two-thirds of the distance from the apex to the base) were placed endocardially using a small trocar to introduce the crystals to the LV cavity. Each crystal was then retracted until slight resistance was felt indicating placement against the endocardial surface. A third crystal was placed on the epicardium of the LV free wall, directly opposed to one of the endocardial crystals. Importantly, the endocardial crystals that is to be used for wall thickness measures must be introduced through the myocardium at an angle so that the final area being measured for wall thickness is not an area that has been subject to trocar introduction. Alignment of crystals to accurately record short axis and wall thickness was confirmed at time of surgery via waveform morphology and oscilloscope signal characteristics. Also, two epicardial pacing electrodes were placed on the right ventricle. Wires from the crystals and pacing electrodes terminated in a skin button and were exteriorized on the back of the animal between the scapulae. Last, an inflatable balloon occluder, sized appropriately for each animal (Access Technologies, Skokie, IL) was secured in place around the thoracic vena cava. The tubing exited the thorax and terminated in a subcutaneous vascular access port on the back of the animal. The thorax was closed in layers, evacuated, and the animal allowed to recover for a minimum of 4 weeks prior to study. Pre-operatively, all animals were administered epidural Morphine (0.1 mg/kg) and Carprofen (4.4 mg/kg, sc). Intra-operatively, all animals were administered Bupivacaine (2.0 mg/kg) locally at the incision site(s). Post-operatively, all animals were administered Carprofen (4.4 mg/kg, po) once a day for 4 days. Animals were assessed for at least 10 days thereafter and were administered additional analgesics as directed by the attending veterinarian.

2.3. Study design

Prior to study, all animals were acclimated to stand in standard canine restraint slings for at least 1 h with stable cardiovascular parameters. Briefly, increasing duration of restraint training with positive reinforcement were performed over several weeks until animals were comfortable remaining in the sling with a HR <110 bpm for 30 min. Following surgical recovery, animals were brought to the lab to assess pressure, ECG, and dimension waveform. Signals with acceptable waveform morphology (shape and magnitude of change) were included in the analysis. RVP at a rate of 240 bpm was initiated for up to 10 days and then reduced to rates ranging from 200 to 220 bpm based on clinical and hemodynamic assessment by or on day 10 of pacing. Thereafter, pacing rate was not altered through the course of the study (a further 2–3 weeks of continuous pacing) to assess stability of dysfunction over time. Basal hemodynamics and cardiac function was assessed in sling-trained conscious beagle dogs prior to the start of pacing (noted as Normal Baseline in the Figures), during the development and progression of cardiac dysfunction: 7 days, 10 days, 14 days, 21 days, 28 days after pacing start (noted as Pacing 7d 10d, 14d, 17d, 21d, and 28d in the Figures). After the 28 day measurement while still subject to constant RV pacing, animals were administered a dose of Vetmedin® (Boehringer Ingelheim) equivalent to pimobendan (0.5 mg/kg, po) and hemodynamics and cardiac function determined 3 h post dose. Statistically significant changes after administration of pimobendan were determined via a paired, one-tail t-test with a $p < 0.05$ considered significant.

All parameters except those that required PV loop data, were determined as the average of 15 min of continuous data for each animal. EF was calculated as the change in end diastolic to end systolic calculated volume divided by end diastolic volume, expressed as a percentage. Similarly, fractional shortening (FS) was calculated as the change in dimension from diastole to systole divided by the end diastolic dimension, expressed as a percentage. Velocity of circumferential fiber shortening was calculated as follows: ((EDD − ESD) / EDD) / ET / $RR^{1/2}$ where

EDD = end diastolic dimension; ESD = end systolic dimension; ET = LV ejection time; and RR = RR interval. LV short axis dimension was used to calculate LV volume in real time using a "cubed" (L^3) method and continuous LV pressure was recorded from the LV pressure probe to generate PV loops. End diastolic and end systolic LV wall stress was calculated as 1.36 (LVP * LVID) / 2 * WT in g/cm^2 (Hittinger et al., 1994; Komamura et al., 1992) using the values of LV pressure (LVP), LV internal diameter (LVID) and wall thickness (WT) at end diastole (just prior to LV contraction) and end systole (at point of LV pressure waveform decay), respectively. To determine basal PV loop data a representative region of at least 10 consecutive loops was identified within the 15 min baseline period and the average data from that region calculated. To determine load-independent parameters of cardiac contractility, transient IVCo (5–10 s per event) was performed in conscious animals after the 15 min baseline assessment and was performed 2–3 times (~5 min between each occlusion) in each animal at each time point by rapid, transient cuff inflation. End systolic elastance (Ees) was determined as the linear slope of the end systolic pressure volume relationship. Preload recruitable stroke work (PRSW) was determined from the linear relationship of stroke work to end diastolic volume during transient IVCo. End diastolic pressure volume relationship (EDPVR) was also calculated as the slope of the linear regression of end diastolic points during IVCo. Typically, the average of at least 2 IVCo sessions was used to determine values for each time point and animal. All data analyzed in Notocord-Hem® (v4.3.0.67, Notocord, Croissy-sur-Seine, France). Continuous RV pacing was suspended approximately one hour prior to hemodynamic assessment and re-started immediately after. Pacing was checked 2×/day and hemodynamics and clinical evaluations were performed at 1–2 times/week throughout the study to ensure consistency of model and animal welfare, respectively.

3. Results

Due to technical failure or morphology of some signals recorded, animals may have been able to be used for certain endpoints, but not all. As such, 5 of 5 animals were used for ECG, arterial and LV pressure recording, 4 of 5 used for those parameters that required LV chamber dimension and/or LV wall thickness. Importantly, we found a critical review of the waveform morphology during surgery, in addition to more traditional oscilloscope-based signal strength assessment, increased the success of obtaining all parameters in all animals to 90% or greater. Fig. 1a is a representative picture of ECG, pressure, LV short axis dimension and LV free wall thickness waveforms 4 weeks post-surgery. Following determination of basal hemodynamics and PV loop data, a more discreet assessment of cardiac contractility via IVCo was completed in conscious beagle dogs to determine end Ees and PRSW (Fig. 1b and Table 1). Similarly we calculated EDPVR to better understand the utility of this parameter with the current instrumentation (Table 1). Despite the challenge in crystal placement to obtain high quality wall thickness measures without a dedicated second pair of crystals, we were able to determine wall thickness changes in 4 out of 5 dogs. In combination with simultaneous LV pressures and diameters, continuous wall stress was calculated. We then identified the points of end diastole and end systole to report end diastolic and end systolic wall stress (Table 1).

In addition to demonstrating the feasibility of conducting detailed CV function in physiologically normal, restrained conscious beagle dogs, we then induced cardiac dysfunction by RVP. This afforded the following advantages: 1) to further characterize the ability to detect changes in cardiac structure and function, 2) to inform on feasibility of using this type of instrumentation in longer term SP models, and last, 3) to provide the foundation for characterization of a model design whereby one could consider risk assessment testing in the setting of cardiac dysfunction. For this, pacing was started at 240 beats/min for up to 10 days at which time pacing was adjusted, one time and per animal based on its level of dysfunction, and then hemodynamics and cardiac function followed for up to 2 additional weeks. The objective was to use the first 10–14 days of pacing as the development phase of dysfunction, then adjust conditions once to maintain relative stability of parameters such that it could potentially afford repeat test agent administration for up to 2 weeks.

Signs of development of systolic dysfunction occurred quickly and were noted in all animals 7 days after pacing as decreases in mean arterial blood pressure (MBP), EF, FS, ±dP/dT, and an increase in tau, the time of isovolumic relaxation (Fig. 2). However, there was not a reflex increase in resting heart rate (HR), nor was there a rise in LVEDP, characteristic of more sustained cardiac dysfunction. Between 7 and 14 days of pacing there was a slight, continued decrease in EF and increase in tau and LVEDP; however, FS, and ±dP/dT remained decreased but similar to day 7 (Fig. 3). Fig. 3A shows the qualitative change in basal PV loops in one animal from baseline to progression to cardiac dysfunction 14 days after initiation of RVP. The latter characterized by a shorter, wider loop that is shifted to the right as compared to baseline. Stroke work (SW), the area of the PV loop, is a straightforward parameter to determine the work of the heart to eject blood. Both qualitatively (as observed in Fig. 3A) and quantitatively (Fig. 2, graph SW) there is a clear progression of decreased SW starting 7 days after pacing that remained relatively stable over the course of pacing. End diastolic and end systolic wall stress also increased from baseline to 14 days of pacing and then

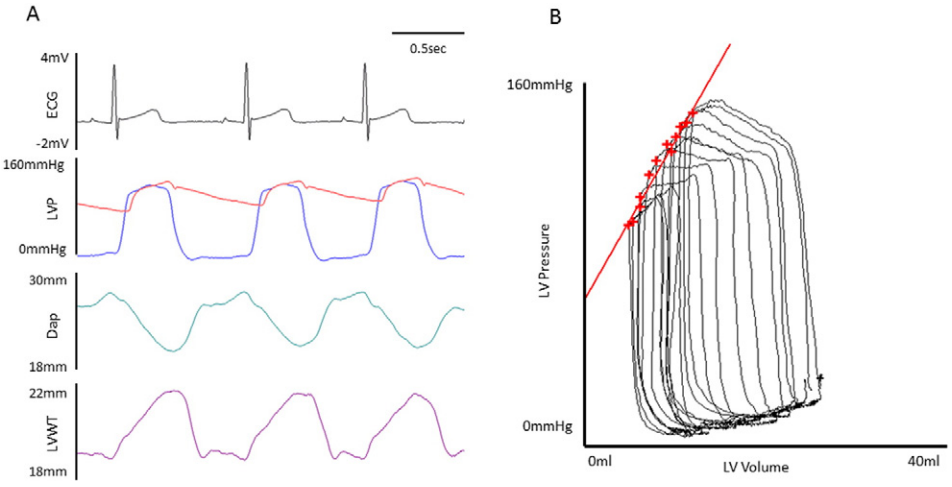

Fig. 1. A. Raw waveforms (in order from top to bottom) for ECG, LV and aortic pressure. LV internal diameter and LV free wall thickness. Dap = anterior-posterior LV chamber diameter. LVWT = LV free wall thickness B. Example of series of PV loop during IVCo at baseline in a conscious beagle dog, line = End systolic pressure volume relationship, the slope of which is Ees.

Table 1
Baseline values in conscious male beagle dogs (n = 5) prior to RVP. MBP mean arterial blood pressure; HR = heart rate; LVSP = LV systolic pressure; +dP/dT or −dP/dT = maximum positive and negative, respectively, first derivative of LV pressure waveform; tau isovolumic relaxation index calculated via the Weiss method; EF = ejection fraction; Vcfc = velocity of circumferential shortening; ESWT − EDWT = end diastolic − end systolic wall thickness; SW = stroke work; Ees = end systolic elastance; PRSW = preload recruitable stroke work; EDPVR = end diastolic pressure volume relationship; QTcVW = QT corrected for heart rate via Van de Water's equation.

Parameter	Mean	SE
MBP (mm Hg)	111	3
HR (bpm)	90	3
LVSP (mm Hg)	148	4
+dP/dT (mm Hg/s)	3709	158
−dP/dT (mm Hg/s)	−4099	68
Tau (weiss, ms)	24.2	0.4
EF (%)	63.0	3.5
Vcfc ($s^{-1/2}$)	1.3	0.1
WT (mm)	3.0	0.7
Wall stress end diastolic (g/cm^2)	9.7	1.42
Wall stress end systolic (g/cm^2)	87.5	8.2
SW (mm Hg∗L)	1.7	0.2
Ees (mm Hg/L)	10911	1653
PRSW (mm Hg)	89.2	1.1
EDPVR (mm Hg/L)	919	144
PR interval (ms)	82.4	4.7
QRS interval (ms)	42.6	4.2
QTcVW interval (ms)	250.0	4.7

remained relatively stable with a further slight increase noted at day 28 of pacing (Table 2). To determine load independent contractility parameters, transient IVCo was performed at baseline (prior to pacing) and on days 14, 17, 21, and 28 of pacing in conscious dogs. Fig. 3B shows a representative PV loop analysis during IVCo at baseline and after 14 days of RVP with a characteristic flattening of the end systolic pressure volume relationship 14 days after pacing. Quantitatively, decreases in both Ees and PRSW were observed from baseline to 14 days of pacing: a characteristic of this model of cardiac dysfunction. Interestingly, there was a slight continued decrease (~25%) in Ees from day 14 to 28 of pacing. However, PRSW remained relatively stable (±10%) over this time and has been suggested as a more stable assessment of contractility (Rahko, 1994; Feneley et al., 1992). EDPVR showed small, but variable increases 3 to 4 weeks after the start of pacing (Fig. 4).

At the end of the assessment of the profile of cardiac dysfunction over time, animals were administered a single dose of pimobendan (0.5 mg/kg, po) (Guth et al., 2015; Markert et al., 2007) and a full assessment of hemodynamics and cardiac function performed 3 h post-dose. As shown in Fig. 5, pimobendan caused a left shift in the PV loop with little change in LV systolic pressure, suggestive of increased contractility combined with afterload reduction. Specifically, administration of pimobendan resulted in a slight but not physiologically significant decreases in MBP. However, pimobendan caused significant decreases in resting HR and tau while there was a physiologically relevant, but not statistically significant, improvement in LVEDP (Fig. 6). Moreover, there were significant increases in +dP/dT, EF, FS, Vcfc, and a non-significant, but likely physiologically relevant, mild increase in SW. In addition, there was a mild increase in LV free wall thickening (Fig. 6, ESWT-EDWT), and a significant decrease in ESD but not EDD (data not shown). Pimobendan administration resulted in slight, but not statistically significant improvement in end diastolic LV wall stress (mean of 22 ± 3 g/cm^2 vs 17 ± 2 g/cm^2 3 h post-dose) with no statistically significant effect on end systolic wall stress (mean of 118 ± 18 g/cm^2 vs 102 ± 11 g/cm^2) 3 h post-dose. Transient IVCo following pimobendan administration demonstrated physiologically relevant increases in both Ees and PRSW and a small, but not significant, decrease in EDPVR (Fig. 7).

4. Discussion

Many previous reports that concentrated on a better assessment of cardiac function in SP studies have focused on LV pressure alone or echocardiography derived parameters. Parameters calculated from the LV pressure waveform and/or echocardiography can be highly informative and have several advantages: surgical instrumentation to acquire

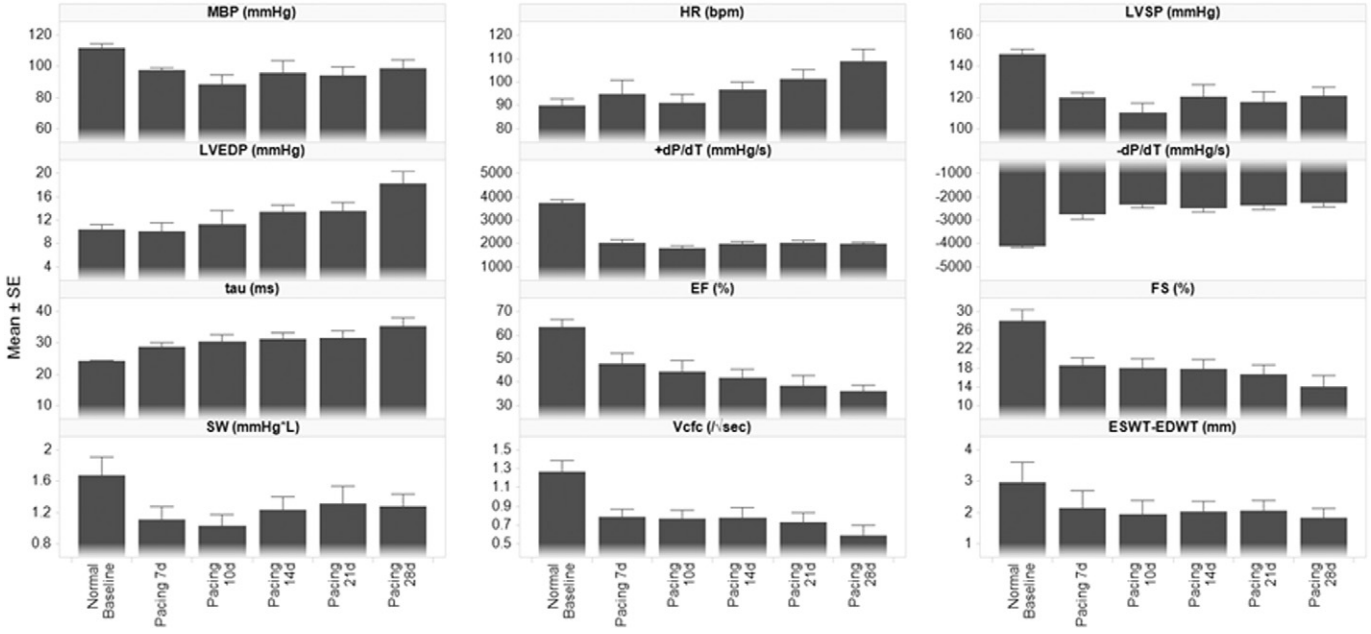

Fig. 2. Progression of changes in various parameters at baseline (normal baseline) and then 7, 10, 14, 21, 28 days (d) after start of continuous RVP in conscious male beagle dogs (n = 4–5). Mean ± SE. MBP mean arterial blood pressure; HR = heart rate; LVSP = LV systolic pressure; posdP/dT or negdP/dT = maximum positive and negative dP/dT, respectively; tau isovolumic relaxation index calculated via the Weiss method; EF = ejection fraction; FS = fractional shortening; SW = stroke work; Vcfc = velocity of circumferential shortening; ESWT − EDWT = end systolic − end diastolic wall thickness.

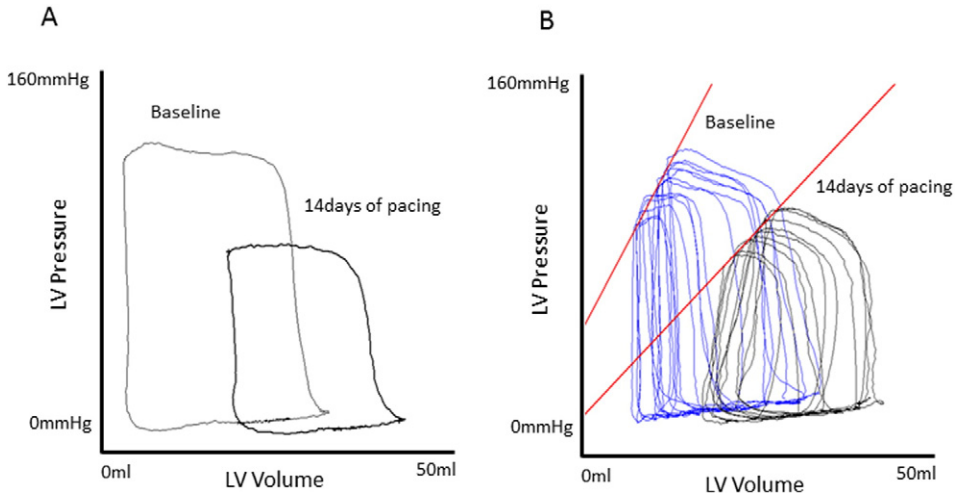

Fig. 3. A. Change in morphology of single PV loop from one beagle dog prior to RVP (baseline) and 14 days after start of continuous RVP. B. Example of series of PV loop during IVCo at baseline and after 14 days of continuous RVP. The qualitative associated change in Ees, the slope of the end systolic pressure volume relationship (line) is also shown.

the waveform is straightforward (or nonexistent in the case of echocardiography), automated calculation of indices such as dP/dT is commonly available within data acquisition software, and telemetry devices are equipped to record the LVP waveform so it can more easily be integrated into existing SP nonGLP or GLP telemetry paradigms. Despite the clear value, dP/dT, for example, and its interpretation can be confounded by changes in heart rate, cardiac preload, and/or cardiac afterload. Individually, some of these can partially be addressed by various "normalization" methods. For example, dP/dT @ 40 mm Hg (the value obtained when LVP reaches 40 mm Hg prior to blood ejection), is generally considered less dependent on afterload, or generating plots of dP/dT vs heart rate (Markert et al., 2012) or dP/dT vs echocardiograph-derived ejection fraction (EF) (Cools et al., 2014) and assessing a shift in the relationship may compensate for some dependencies.

In part because of the abovementioned dependencies, the PV loop, particularly in settings without the confound of anesthesia, remains the "gold standard" for cardiac function assessment. The ability to measure PV loops in canines (mongrel dogs) via various methods has existed for decades (Little et al., 1989; Sodums et al., 1984; Suga et al., 1973; Sagawa, 1981; Kass et al., 1987) and is still used in a limited number of laboratories (ex: Shen et al., 2010). However, these techniques applied to conscious SP approaches seem to have been largely "lost" to the safety pharmacologist. Perhaps due to in part to the standard instrumentation techniques that are not amenable to telemetry and that have traditionally required significant, chronic post-surgical maintenance. Also the canine models using these endpoints are almost exclusively conducted in mongrel dogs vs beagle dogs, the more commonly used strain for nonGLP/GLP SP and toxicology studies. However, as SP is asked to blend regulatory science with more investigative approaches such techniques can add value by providing key unambiguous comprehensive data on cardiovascular function, to deliver key decision enabling data at various stages of drug development. Therefore, a "resurfacing" of such techniques is warranted.

Table 2.
Effect of RVP on end diastolic (ED) and end systolic (ES) LV free wall stress in conscious male beagle dogs (n = 4). Mean ± SE.

Time point	WallStressEDP		WallStressES	
	Mean	SE	Mean	SE
Normal baseline	9.7	1.4	87.5	8.2
Pacing 14d	15.3	2.5	101.7	12.4
Pacing 17d	15.8	1.5	104.7	19.1
Pacing 21d	15.6	2.4	106.9	15.9
Pacing 28d	21.5	3.2	117.7	17.9

To this end, we characterized conscious PV loop studies in a chronically instrumented beagle dog model. While there is not yet a readily available solution for telemetered cardiac dimension/volume data with integrated pressure measurement, the ability to "internalize" some signals (arterial and LV pressures and ECG) via telemetry and integrate the sonomicrometry crystals into a titanium skin button decreased the maintenance and failure rates and increased the long-term use of the animal. Taken together this provided a model whereby arterial pressure, LV pressure, LV dimension, LV wall thickness, and ECG could be simultaneously recorded in animals with minimal maintenance. Further such a model could serve a dual role: used for telemetry studies (BP, LVP, ECG) and when needed restrained studies to investigate more detailed endpoints of cardiac structure/function along with hemodynamics. The value of such an approach can be significant, for not just single dose studies, but also for determination of combined hemodynamics and cardiac function/structure following sustained exposure of test agents. For example the preclinical physiologic mechanism of the myosin activator, Omecamtiv, currently in clinical development for heart failure was not an overt change in peripheral hemodynamics or + dP/dT, but rather an effect on parameters not routinely measured (Shen et al., 2010). While this is an example of a desired effect, it is easy to theorize the potential where changes in CV function could have meaningful risk implications but not be manifest in overt changes in peripheral hemodynamics or more common LV indices.

There is no guidance for use of disease models in Safety Pharmacology. It is clear that studies in disease models to assess potential risk must necessarily be approached with caution given many functional challenges, including but not limited to, increased inter-animal variability. As well, one must have an understanding of the clinical translatability of the model and its relevance to effectively assess the potential on- or off-target activity. Despite these hurdles, there can be value in a better understanding of test agent-dependent effects in pathophysiologic conditions. There are relatively few cardiac disease models reported in beagle dogs, including those that describe RVP (Watanabe, Kuroda, Sato, & Makino, 2006; Kataoka et al., 2001). To better understand the value of the well-known method of RVP to create systolic dysfunction/dilated cardiomyopathy in beagle dogs, we characterized the development and maintenance of cardiac dysfunction for up to 28 days of RVP. Previous papers describing the use of RVP in beagle dogs reported varied initial pacing rates of 220 to 260 beats/min which were maintained at that level for up to 4 weeks, but there was little information on the development/progression of the cardiac dysfunction (Watanabe et al., 2006; Kataoka et al., 2001). We found a RVP pacing rate of 240 beats/min, commonly used in mongrel dog RVP models (Regan et al., 2008; Shen et al., 2010), maintained for a minimum of 7 days was adequate

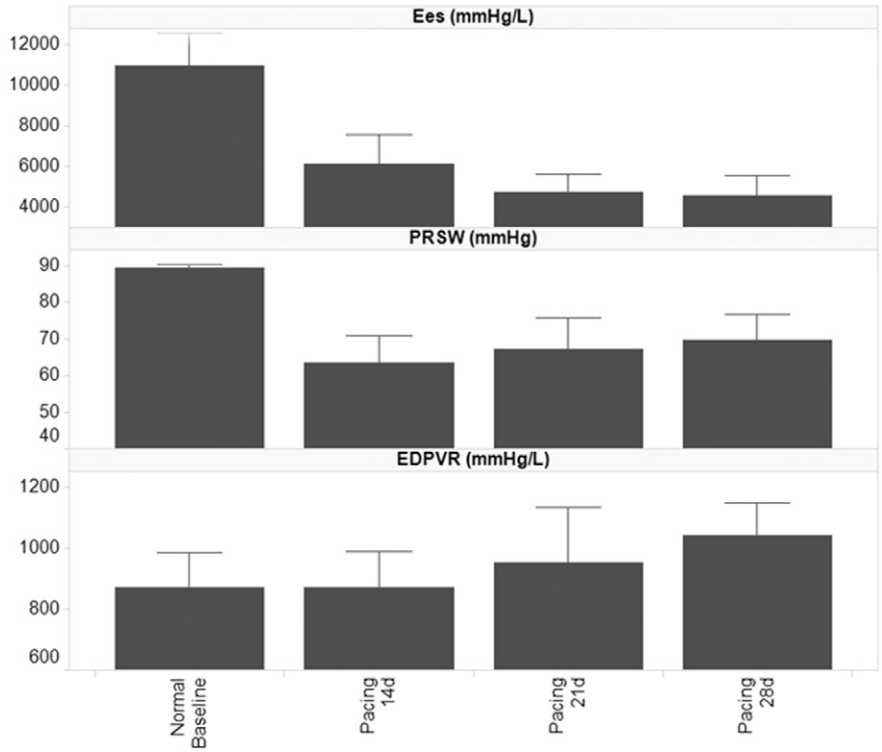

Fig. 4. Changes in load independent cardiac contractility as assessed by end systolic elastance (Ees) and preload recruitable stroke work (PRSW) and well as the profile of the end diastolic pressure volume relationship (EDPVR) 14, 21, 28 days (d) after start of continuous RVP in conscious male beagle dogs (n = 4). Normal baseline = values obtained prior to start of RVP.

to consistently create a level of dysfunction in all animals. However it was critical to evaluate dogs early in the pacing protocol, and adjust pacing rates for each dog, as determined by state of dysfunction, to reduce variability among animals. This also identified an optimized RVP rate for each dog that could be unchanged over a course of time (up to 14 days in this characterization) to maintain a relatively stable dysfunction. We then administered pimobendan after 28 days of continuous RVP. Pimobendan is a well-known inodilator: increasing cardiac contractility and inducing arterial vasodilation. Its effects on peripheral hemodynamics and LV pressures in normal beagle dogs has been well-described (Guth et al., 2015; Norton et al., 2009). After establishment of cardiac dysfunction, both mechanisms of action of pimobendan could

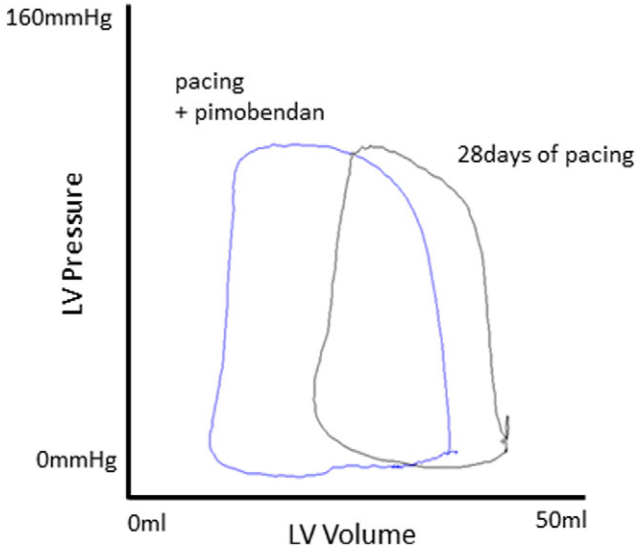

Fig. 5. Example of the effect of pimobendan (0.5 mg/kg, po) on the morphology of a single PV loop following 28 days of RVP.

be readily detected by a leftward shift in the PV loop without an increase in LVSP. We also observed improvement in Ees and PRSW, two load independent indices of cardiac contractility. Together this series of studies demonstrated the ability of the model and instrumentation to not only detect decreases in cardiac function (as evidence by the ability to detect progression of disease), but also improvement of cardiac function in beagle dogs with underlying cardiac dysfunction. Further it provided a method by which relatively stable dysfunction could be obtained without altering pacing requirements. Importantly, this requirement would only be needed in studies where one endeavored to utilize a repeat dose strategy to evaluate test agent dependent effects over time as the reduction in variability afforded would increase the confidence of results. However, study designs targeted to determine the short-term effect of test agents in dogs with underlying cardiac dysfunction only need to be focused on maintaining a consistent level of dysfunction among animals for a shorter time. For these types of studies, one could envision a paradigm where pacing rates are more freely adjusted, even turned off between studies, thus creating increased flexibility and decreased variability. Further it is tempting to speculate that turning off the pacing and allowing animals to return to baseline levels may enable a chronic colony of "on demand" cardiac dysfunction for testing only when needed, although such an approach would require significant characterization.

One potential confound of such a model is the wealth of data it generates; not only in direct parameters determined from the various waveforms, but also the numerous possible calculated values and relationships between variables – all with varying utility to describe test agent dependent effects and mechanism of action. As such, care must be taken to utilize key endpoints effectively and understand limitations of all potential endpoints. For this initial characterization we chose to focus on a restricted set of data for analysis and to instrument animals with one set of LV dimension crystals along the anterior-posterior LV short axis. We are aware of previous papers that utilized multiple axes; however, those often involved significantly larger mongrel dogs and/or models where localized wall motion abnormalities make it critical for additional LV dimensions, or study objectives where a higher

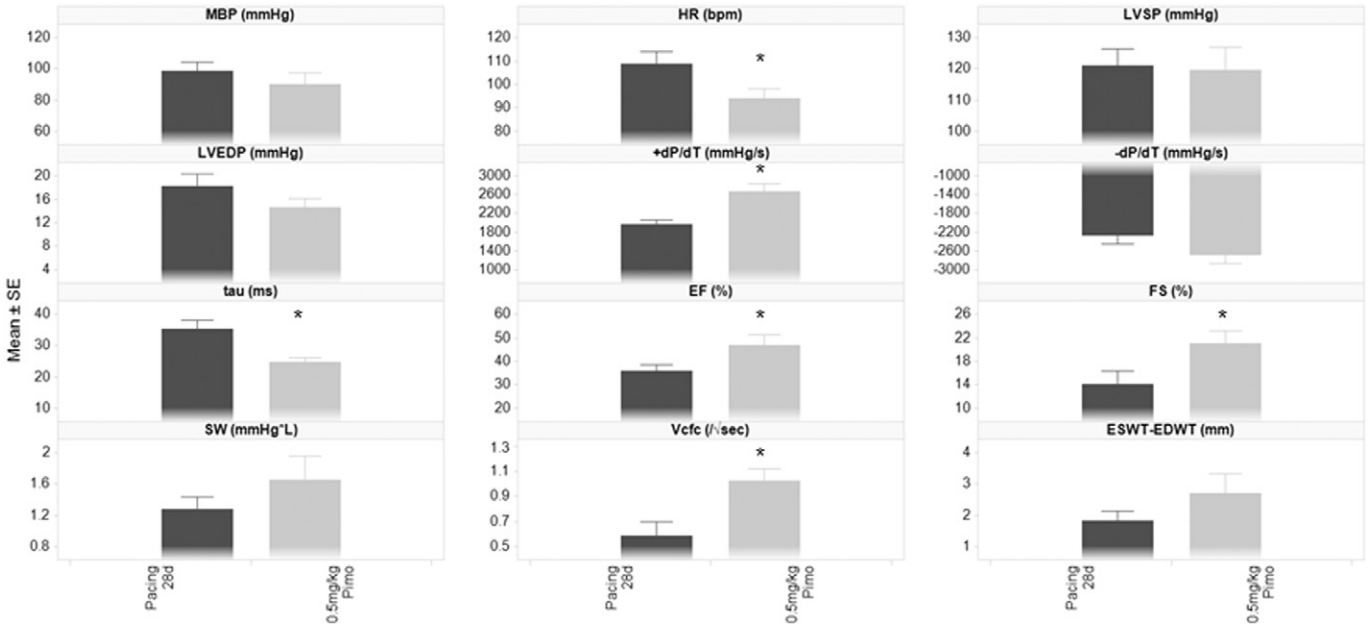

Fig. 6. Effects of pimobendan (0.5 mg/kg, po) on various cardiovascular parameters after 28 days (d) of RVP in conscious male beagle dogs (n = 4). Mean ± SE. Dark bars = 28 days of RVP; light bars = 3 h post 0.5 mg/kg, po pimobendan. *$p < 0.05$ via paired *t*-test.

resolution of change dimension along multiple axes was required. While additional LV axes in this study would have undoubtedly improved the calculation of true LV volume, we were focused primarily on changes in global systolic function/contractility in normal animals where a single dimension can be adequate (Suga and Sagawa, 1974; Little et al., 1985), and then we induced RVP to create global, not regional, LV structure and function changes. Moreover, a single diameter determination of volume resulted in acceptable error (<10%) in mongrel dogs (Suga and Sagawa, 1974; Little et al., 1985). Of note, a comparison of EF values obtained with echocardiography conducted immediately after our CV assessment showed very similar magnitude of change over time albeit with different absolute values, owing in part to the different methods of calculation (data not shown). Also, our calculated end diastole and end systole volumes (data not shown) were in line with previous reports in beagle dogs before and after RVP calculated by pulmonary arteriography (Kataoka et al., 2001). We further used one of the

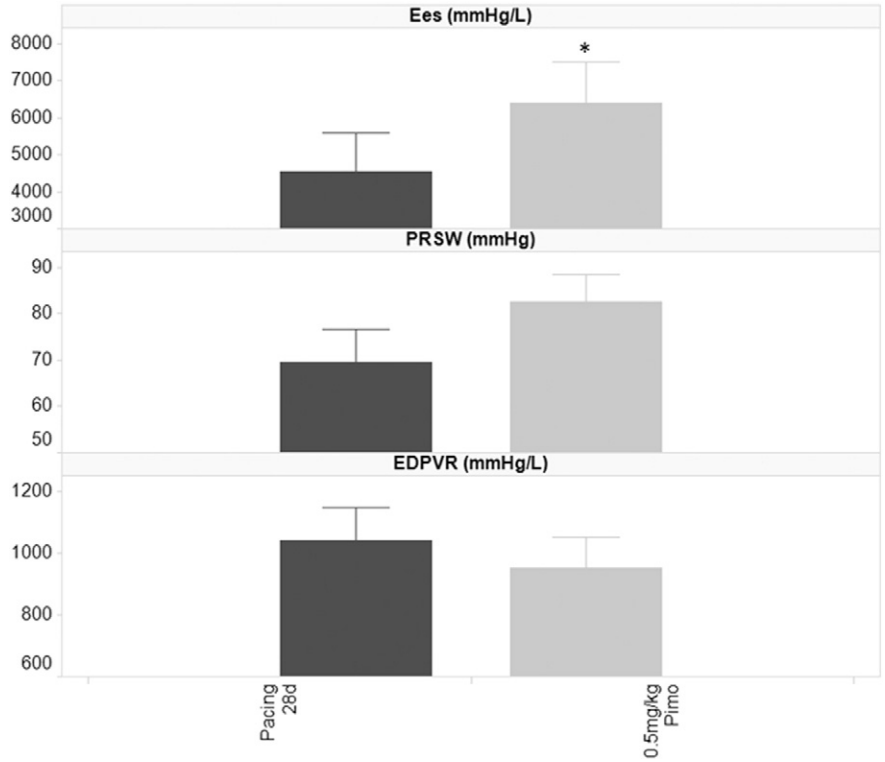

Fig. 7. Effects of pimobendan (0.5 mg/kg, po n = 4) on load independent contractility as assessed by end systolic elastance (Ees) and preload recruitable stroke work (PRSW) and well as the effect on the end diastolic pressure volume relationship (EDPVR) after 28 days (d) of RVP. Mean ± SE. Dark bars = 28 days of RVP; light bars = 3 h post pimobendan (0.5 mg/kg, po). *$p < 0.05$ via paired *t*-test.

endocardial crystals to also determine LV wall thickness by placing a third crystal on epicardial surface of the LV free wall, aligned with the endocardial crystal. The fixed positions of the probes provided very high resolution method to determine the degree of change. Other methods, such as echocardiography, where resolution and probe placement over repeat measures are more challenging may result in increased variability/reduced sensitivity. It is well-known that LV wall stress, for which LV wall thickness is one critical variable, is a key component of cardiac oxygen consumption (Strauer, 1979); a potentially valuable endpoint in assessing cardiovascular risk, so high resolution data on wall thickness could be a valuable parameter.

Taken together, the data show the feasibility in measuring direct concomitant cardiac structural and functional parameters in normal beagle dogs and further describe conditions whereby relatively stable cardiac dysfunction can be maintained over a time course to assess repeated dose effects. The former would enable the safety pharmacologist to conduct deeper, second tier assessment in a chronically instrumented dog model and (if desired) combined with the latter, could allow for risk assessment in the setting of underlying cardiac dysfunction. It must be noted that additional caution must be applied to fully understand the advantages, limitations, and relevance of the pathophysiology of any disease model in the setting of safety risk assessment. Ultimately, the in vivo safety pharmacologist needs to be aware of the feasibility of such techniques, to weigh advantages and limitations of the various additional instrumentation options, and as needed to apply the appropriate range of such cardiovascular assessments, from straightforward BP and ECG through to more complex, comprehensive readouts of cardiac and vascular function to enable drug development through the conduct of investigative safety pharmacology studies.

Conflict of interest statement

All authors are employees of Merck and Co., Inc.

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