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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