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An evaluation of the utility of LVdP/dt$_{40}$, QA interval, LVdP/dt$_{min}$ and Tau as indicators of drug-induced changes in contractility and lusitropy in dogs

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ABSTRACT

Introduction: The importance of drug-induced effects on the inotropic state of the heart is well known. Unlike hemodynamic and cardiac electrophysiological methods, which have been routinely used in drug safety testing for years, the non-clinical assessment of drug effects on myocardial contractility is used less frequently with no established translation to humans. The goal of these studies was to determine whether assessment of alternate measures of cardiac inotropy could detect drug-induced changes in the contractile state of the heart using drugs known to have clinically relevant positive and negative effects on myocardial contractility. This study also evaluated drug-induced effects on lusitropy (relaxation) parameters of the heart.

Methods: A double 4 × 4 Latin square study design using Beagle dogs (n = 8) was conducted. Drugs were administrated orally. Arterial blood pressure (BP), left ventricular pressure (LVP) and the electrocardiogram (ECG) were assessed across different laboratories using the same protocol. Each of the six laboratories studied at least 2 drugs (one positive inotrope (pimobendan or amrinone) and one negative inotrope (itraconazole or atenolol) at 3 doses selected to match clinical exposure data and a vehicle control). Animals were instrumented with an ITS telemetry system or DSI's D70-PCTP or PhysioTel™ Digital system. The data acquisition and analysis systems used were Ponemah, Notocord or EMKA.

Results: The derived inotropic and lusitropic parameters evaluated included peak systolic and end diastolic LVP, LVdP/dt$_{max}$, LVdP/dt$_{40}$, QA interval, LVdP/dt$_{min}$ and Tau. This study showed that LVdP/dt$_{40}$ provided essentially identical results to LVdP/dt$_{max}$ qualifying it as an index to assess drug effects on cardiac contractility. LVdP/dt$_{40}$ provided an essentially identical assessment to that of LVdP/dt$_{max}$. The QA interval did not react sensitively to the drugs tested in this study; however, it did detect large effects and could be useful in early cardiovascular safety studies. The lusitropic parameter, LVdP/dt$_{min}$, was modestly decreased, and Tau was increased, by atenolol and itraconazole. At the doses tested, amrinone and pimobendan produced no changes in LVdP/dt$_{min}$ while Tau was modestly increased. The drugs did not produce effects on BP, HR or the ECG at the doses tested. Blood samples were drawn to confirm drug exposures predicted from independent pharmacokinetic studies.

Discussion: These findings indicate that this experimental model can accurately and consistently detect changes in cardiac contractility, across multiple sites and instrumentation systems. While LVdP/dt$_{40}$ produced responses similar to LVdP/dt$_{max}$, the QA interval and lusitropic parameters LVdP/dt$_{min}$ and Tau were not markedly changed

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at the dose of drugs tested. Further studies with drugs that affect early diastolic relaxation through calcium handling are needed to better evaluate drug-induced changes on lusitropic properties of the heart.

1. Introduction

The maximum rate of rise of left ventricular pressure ($LVdP/dt_{max}$) was proposed for use as an index of cardiac contractility in preclinical drug testing in 2011 with the caveat that it could be shown to provide similar data describing drug-induced effects when evaluated across multiple laboratories (Sarazan et al., 2011). If the model is to be useful in predicting risk to patients, the relationship between plasma drug concentration and its inotropic effect in any preclinical animal species should also be similar in that preclinical model used to that seen in humans. With these goals in mind, the International Life Sciences Institute (ILSI) Health and Environmental Sciences Institute (HESI) organized a multi-center consortium and the first series of study results confirmed that $LVdP/dt_{max}$ could be used reliably across laboratories and that its dose/exposure-response to drug-induced changes seemed to parallel very closely to that seen with their clinical use (Guth et al., 2015). In that evaluation, study participants designed a double 4 × 4 Latin square study (n = 8/site) using chronically instrumented Beagle dogs to assess dose-dependent LV contractility changes after treatment with drugs known to have clinically relevant positive (pimobendan and

Table 1
Contractility effects of positive and negative inotropic drugs tested in conscious telemetered Beagle dogs.

Test article	# Drug testing sites	Doses (mg/kg)	Formulation/Vehicle
Positive inotrope			
Pimobendan	3	Vehicle, 0.1, 0.3, 1.0	PCCA Fixed Oil Suspension Vehicle™
Amrinone	5	Vehicle, 0.5, 2.0, 5.0	Gelatin capsules
Negative inotrope			
Itraconazole	4	Vehicle, 3, 10, 30	0.5% (w/w) Methocel E50 (in H_20 + 0.01% (w/w) Polysorbate 80 + 10 mM Phosphate Buffer (pH 6.80–7.20)
Atenolol	3	Vehicle, 0.3, 1.0, 3.0	Deionized water

Positive and negative inotropic drugs were administered orally to Beagle dogs (n = 8 per group) at the doses in the Table using the formulation/vehicle described (Ahmad, Singer, & Leiss, 2001; Alousi & Johnson, 1986; Chu, Hu, & Shieh, 1999; de Abreu, de Castro, & Pedrazzoli, 2003; Hardin et al., 1988; Kato, 1997; Kullberg et al., 1981).

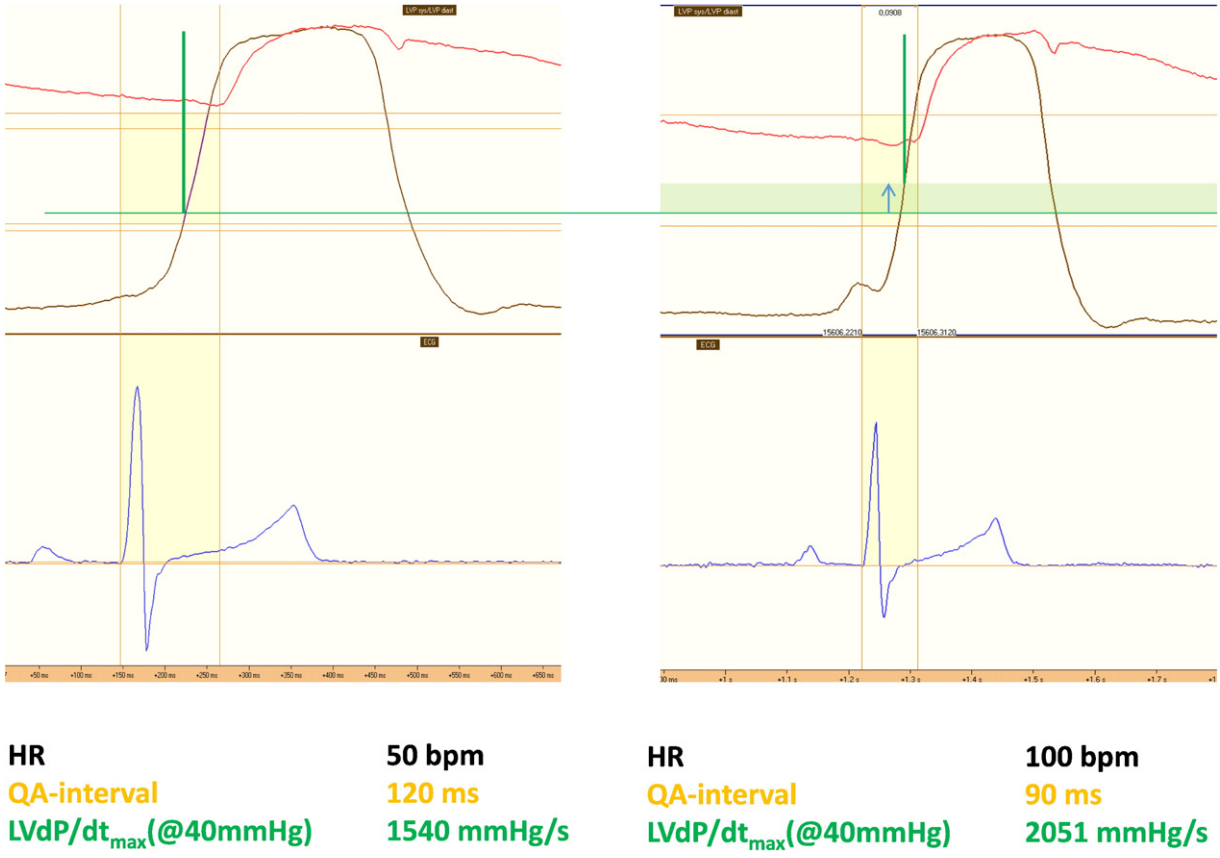

Fig. 1. Measurement of the QA interval and $LVdP/dt_{40}$ in a conscious Beagle dog. The QA interval (shaded in yellow) is measured from the onset of the Q-wave of the ECG (lower panel) to the onset of the upstroke of the aortic BP pulse (upper panel, red line). $LVdP/dt_{40}$ (or $LVdP/dt_{max}$ at 40 mmHg) is delineated by the green lines (shown in the upper panels) and measured from the LV pressure trace (brown trace in the upper panel). Both QA and $LVdP/dt_{40}$ are shown as measured at a HR of 50 bpm (left panels) and at 100 bpm (right panels). Note figure is not to scale.

Table 2
Characteristics of the study sites and drugs tested in all contractility assessment studies at participating companies.

Participant	Sex	Source	Telemetry system	Software system
AbbVie	M	Marshall Farms	DSI PhysioTel™ *D70-PCTP*	EMKA
Amgen/Covance	M	Covance Research Products	DSI PhysioTel™ *D70-PCTP*	Ponemah
Boehringer Ingelheim	M/F	Marshall/Boehringer Ingelheim	Konigsberg (*ITS*) *T27*	Notocord
Data Sciences International (DSI)	M	Covance Research Products	DSI PhysioTel™ Digital L21	Ponemah
Millennium	M	Marshall BioResources	DSI PhysioTel™ *D70-PCTP*	EMKA
Sanofi	M/F	Harlan	DSI PhysioTel™ *D70-PCTP*	Notocord

Participating companies are listed alphabetically. The transmitters used contain multiple pressure catheters that may be used to measure parameters such as systemic pressure and left ventricular pressure (LV pressure). Beagle dogs were used at all study sites. M = male; F = female.

Table 3
An outline of the study super-intervals constructed for each test article.

Positive Inotrope		Negative inotrope	
Pimobendan	Amrinone	Atenolol	Itraconazole
0–6	0.5–3.5	0.5–5.5	0.5–3.6
8–13	5.0–6.5	8–12.17	5–6
13–18	8–13.5	12.17–16.5	8–13
20–24	13.5–19	20–24	13–18
	20.5–24		20–24

The super-intervals define the hours after dosing and were constructed for each test article prior to statistical analysis in order to avoid recording disturbances (i.e., from dosing or blood sample collection or changes in light cycle). See Guth et al. (2015) for details.

amrinone) or negative (itraconazole and atenolol) inotropic effects (Table 1). Additionally, arterial BP, LVP and the ECG were collected using telemetry prior to dosing and over a 24 h period post-dose. In the first report from those studies (Guth et al., 2015), parameters derived in addition to LVdP/dt_{max} included peak systolic LVP and end-diastolic left ventricular pressure (LVEDP), heart rate (HR) as well as arterial BP. Additional derived parameters have been calculated from those studies but have not yet been reported by this consortium. Two of these parameters used to quantify the inotropic state of the heart include LVdP/dt_{40} (the first derivative of the left ventricular pressure signal at a developed LV pressure of 40 mmHg) (Mason, Braunwald, Covell, Sonnenblick, & Ross, 1971) and the QA interval (the time interval between the onset of the QRS complex and the onset of the aortic blood pressure pulse) (Cambridge & Whiting, 1986) have now been evaluated from conduct of this study (Fig. 1). Furthermore, the lusitropic endpoints (LVdP/dt_{min} and Tau) were calculated from those data and are reported.

Although the data derived from the study indicated that LVdP/dt_{max} was a robust parameter for assessing both clinically relevant drug-induced positive and negative inotropic effects (Guth et al., 2015), its dependency on both preload and afterload (Suga, Sagawa, & Shoukas, 1973; Zimmer, 2001) has been viewed as a potential disadvantage (Hamlin & del Rio, 2012). However, the four drugs selected, at the doses administered, did not produce significant changes in either left ventricular preload or afterload and this may have contributed to the outcome from the study that LVdP/dt_{max} provided a robust, highly sensitive and reliable measure of contractility. The parameter, dP/dt_{40}, is another measure of systolic function. It is the rate of pressure developed in the left ventricle at 40 mmHg and provides an independent, accurate and practical measure of ventricular contractility essentially independent of preload and afterload that is reproducible in both non-clinical and clinical studies (Mason et al., 1971). Therefore, it was decided to evaluate this parameter, in addition to LVdP/dt_{max}, to determine whether it offers any advantages as an index of contractility.

Accurate measurement of left ventricular pressure requires a high fidelity recording system with appropriate expertise in establishment, analysis and interpretation of the findings. Thus, alternative parameters, such as the QA interval, have been proposed and assessed for sensitivity to changes in the myocardial contractile state and do not require a LV pressure signal (Cambridge & Whiting, 1986; Norton, Iacono, & Vezina, 2009).

The QA interval is a simple, selective measure of changes in cardiac contractility whereby reductions in the interval are associated with an increase in contractility and increases in this interval are associated with a decrease in contractility (Adeyemi et al., 2009; Johnson, Geys, Lissens, & Guns, 2012; Sarazan, 2014). Note that Hamlin and del Rio (2010) review the nature of the QA interval and stress that there is a limitation to its use an index of myocardial contractility since it depends upon many physiological factors. We have used the above mentioned

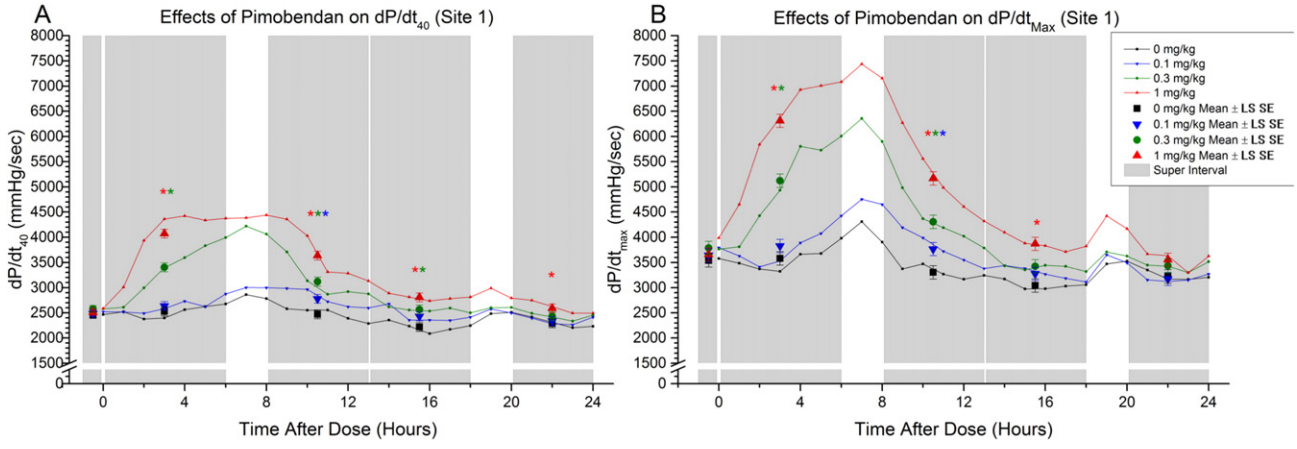

Fig. 2. A comparison of the positive inotropic response of LVdP/dt_{40} compared to LVdP/dt_{max} after administration of pimobendan (from site 1) in conscious instrumented dogs. Pimobendan (vehicle = □, 0.1 mg/kg = ∇, 0.3 mg/kg = ●, 1 mg/kg = Δ) was administered orally at time = 0. The small symbols represent the mean value from the previous 10-min while shaded areas represent super-intervals as defined in Table 3. Large symbols represent the mean ± SE within each super-interval.

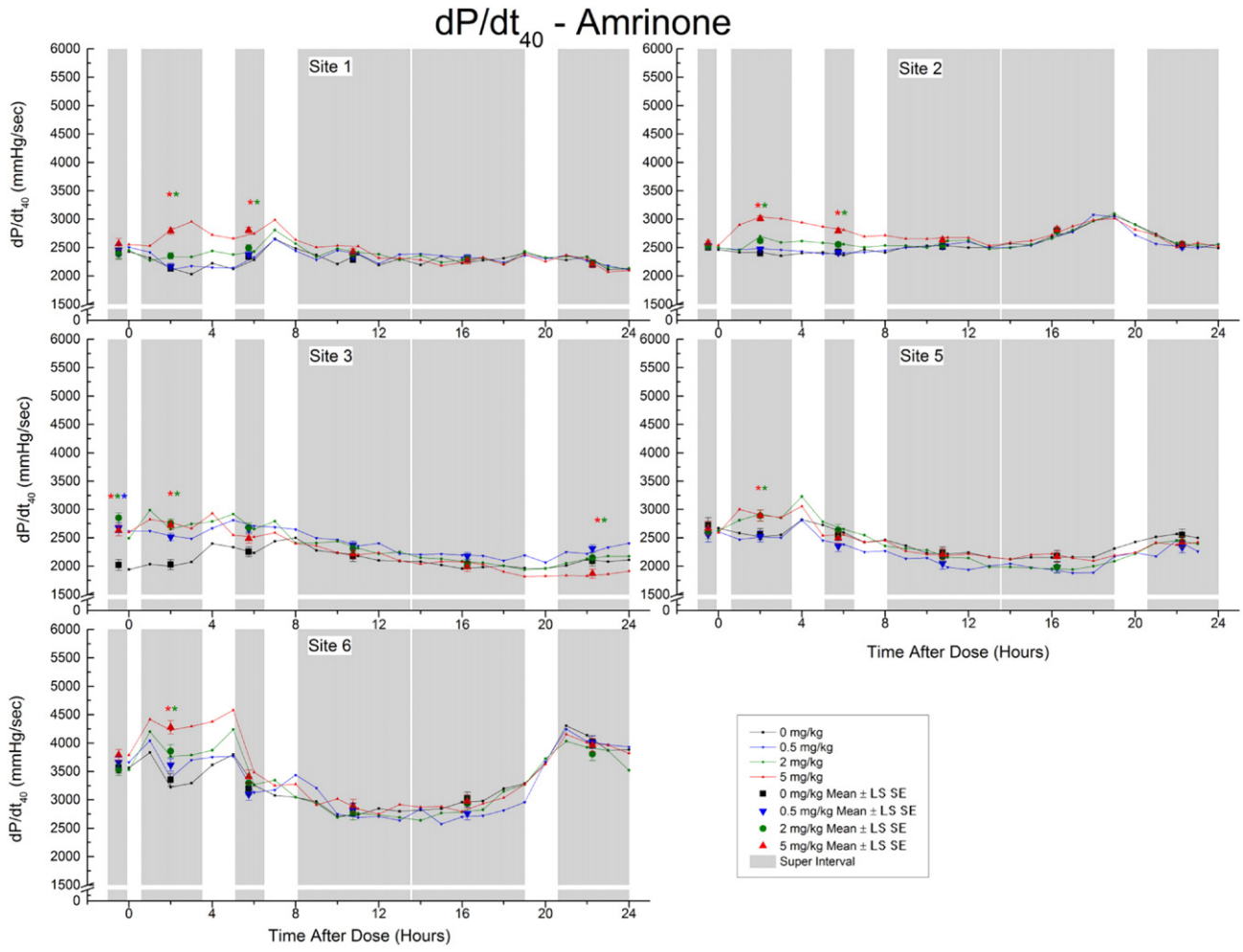

Fig. 3. The effect of amrinone on LV dP/dt_{40} in conscious instrumented dogs at five different laboratories (sites 1, 2, 3, 5 and 6). Amrinone (vehicle = □, 0.5 mg/kg = ∇, 2 mg/kg = ●, 5 mg/kg = Δ) was administered orally at time = 0. The small symbols represent the mean value from the previous 10-min while shaded areas represent super-intervals as defined in Table 3. Large symbols represent the mean ± SE within each super-interval.

raw data to evaluate the performance of the QA interval when compared to dP/dt_{max} and dP/dt_{40} in this study using the conscious Beagle dog.

The importance of drug-induced effects on the inotropic state of the heart has been recognized for some time, but the possible drug-induced effects on the relaxation of the left ventricle, the lusitropic characteristics of the heart, have been recognized only more recently. Since myocardial relaxation is an important physiological component of ventricular systole both inotropy and relaxation are intimately related (Hamlin & del Rio, 2010). An impairment of ventricular relaxation can contribute to a reduced or slowed rate of LV pressure reduction which can have a substantial impact on overall ventricular pump function and cardiac output and this property may be affected by drugs (Gillebert & Raes, 1994; Zile & Gaasch, 1991). Slowed or incomplete relaxation, regardless of the cause, reduces ventricular filling function which can substantially limit cardiac output adaptation to changes in loading conditions (Zile & Gaasch, 1991). Although the four test articles used in this study were selected primarily for their inotropic effects, their effects on two indices of the lusitropic state, $LVdP/dt_{min}$ and Tau, were also assessed.

$LVdP/dt_{min}$ represents the steepest downward (negative) slope of the LV pressure-time curve, reflecting the maximal decay of chamber pressure as the left ventricle relaxes. Similarly, Tau is a calculation of the left ventricular diastolic time constant for isovolumetric left pressure decay (Berne & Levy, 1977; Raff & Glantz, 1981) and has been modeled with various formulae (Bai & Wang, 2010). For the present study, Tau was calculated using the asymptotic, non-null pressure formula (Raff & Glantz, 1981).

The purpose of the evaluation conducted in this study, using a standard safety pharmacology dog implanted with telemetry equipment, was to demonstrate whether or not $LVdP/dt_{40}$ could be used as a reliable index of ventricular contractility compared to $LVdP/dt_{max}$. The performance of the QA interval, in contrast to $LVdP/dt_{max}$ and $LVdP/dt_{40}$, as a surrogate measure of contractility was also assessed as it is not commonly conducted in standard safety studies. The effects of amrinone, pimobendan, atenolol and itraconazole were also assessed on the lusitropic parameters, $LVdP/dt_{min}$ and Tau. While the primary goal of the ILSI/HESI consortium was not to evaluate the lusitropic properties of the drug selected they may be used by safety pharmacologists to evaluate changes in cardiac function since drugs may also have a distinct effect on ventricular relaxation (lusitropy), independent from the contractile state.

2. Materials and methods

2.1. Test facilities

All studies were performed by 6 independent companies. Each of these 6 companies conducted the in-life phase of their study either within their own vivarium or had it outsourced to a contract research

Table 4
The Least Square (LS) mean direct and derived baseline contractility and hemodynamic values for each study site at the pre-dose super-intervals for both positive (A) and negative (B) inotropic drugs.

A - Positive inotropic drugs

Amrinone						Pimobendan		
Parameter	Site 1	Site 2	Site 3	Site 5	Site 6	Site 1	Site 2	Site 4
SBP	135 ± 1.4	154 ± 0.5	145 ± 2.1	107 ± 3	160 ± 1.4	133 ± 0.4	157 ± 0.6	142 ± 0.1
DBP	87 ± 0.9	83 ± 0.3	74 ± 0.9	82 ± 2.8	91 ± 0.9	85 ± 0.6	83 ± 0.5	81 ± 0.3
HR	87 ± 0.8	101 ± 1.0	86 ± 1.5	99 ± 2.6	112 ± 2.3	91 ± 0.8	107 ± 1.9	116 ± 2.0
LVSYS	NR	116 ± 0.5	128 ± 0.8	96 ± 2.3	136 ± 1.8	NR	121 ± 0.7	142 ± 0.7
LVEDP	14 ± 0.2	10 ± 0.2	5 ± 0.4	2 ± 1.4	15 ± 0.5	14 ± 0.2	11 ± 0.2	11 ± 0.2
dP/dt_{max}	3610 ± 72	2822 ± 21	3398 ± 147	3096 ± 77	4684 ± 95	3658 ± 50	3187 ± 31	4567 ± 44
dP/dt_{40}	2453 ± 40	2532 ± 17	2545 ± 180.9	2640 ± 36.9	3637 ± 59	2508 ± 26	2688 ± 28	3305 ± 40
QA	84 ± 0.8	116 ± 0.2	55 ± 1.8	63 ± 3.3	99 ± 0.9	79 ± 0.4	105 ± 0.5	116 ± 1.3
dP/dt_{min}	−3382 ± 46	−2774 ± 9.9	−3360 ± 163	−2417 ± 55.7	−3968 ± 64	−3479 ± 26	−3023 ± 25	−4057 ± 30
Tau	29 ± 0.2	24 ± 0	19 ± 0.6	13 ± 0.2	30 ± 0.4	29 ± 0.2	23 ± 0.1	22 ± 0.1

B - Negative inotropic drugs

Atenolol				Itraconazole			
Parameter	Site 1	Site 2	Site 5	Site 1	Site 3	Site 4	Site 6
SBP	139 ± 0.7	159 ± 1	110 ± 3.7	140 ± 0.6	130 ± 1.1	145 ± 0.9	157 ± 1.6
DBP	90 ± 0.6	84 ± 0.8	87 ± 3.7	89 ± 0.7	74 ± 0.5	81 ± 0.4	88 ± 1.0
HR	96 ± 0.6	96 ± 1.4	104 ± 2.7	86 ± 2.2	89 ± 0.4	112 ± 0.4	96 ± 1.1
LVSYS	NR	123 ± 1.1	98 ± 3.1	NR	131 ± 0.3	144 ± 0.9	127 ± 1.2
LVEDP	15 ± 0.4	13 ± 0.2	4 ± 2.1	16 ± 0.3	4 ± 0.3	13 ± 0.2	16 ± 0.6
dP/dt_{max}	3820 ± 35	2406 ± 58	3015 ± 44	3846 ± 34	3778 ± 18	4894 ± 101	4209 ± 59
dP/dt_{40}	2475 ± 17	2502 ± 24	2485 ± 68	2562 ± 39	NR	3394 ± 54	3255 ± 175
QA	81 ± 0.3	108 ± 0.5	NR	79 ± 0.3	53 ± 0.3	111 ± 1.1	98 ± 0.4
dP/dt_{min}	−3581 ± 21	−2955 ± 28	−2397 ± 35	−3574 ± 44	−3268 ± 55	−4164 ± 68	−3510 ± 44
Tau	29 ± 0.4	16 ± 0.1	13 ± 0.2	30 ± 0.4	17 ± 0.2	22 ± 0.5	33 ± 0.8

SBP = systolic blood pressure; DBP = diastolic blood pressure (mmHg); HR = heart rate (beat per min, bpm); LVSYS = left ventricular systolic pressure; LVEDP = left-ventricular end-diastolic pressure; see text for definitions regarding inotropic (dP/dt_{max}, dP/dt_{40} and QA) and lusitropic (dP/dt_{min} and Tau) parameters; NR = not reported. Note values for pimobendan dP/dt_{min} are from sites 1 and 4 while values for atenolol dP/dt_{max} are from sites 1, 2, and 5. The data are the average of the 4 dose level baseline LSMean values with corresponding standard errors.

DBP = diastolic blood pressure (mmHg); SBP = systolic blood pressure; HR = heart rate (beat per min, bpm); LVSYS = left ventricular systolic pressure; LVEDP = left-ventricular end-diastolic pressure; see text for definitions regarding inotropic (dP/dt_{max}, dP/dt_{40} and QA) and lusitropic (dP/dt_{min} and Tau) parameters; NR = not reported. The data are the average of the 4 dose level baseline LSMean values with corresponding standard errors.

laboratory of their own choosing (Table 2). Each individual study was therefore subject to the local guidelines in terms of the vivarium conditions, study conduct and animal use approval procedures. All participating institutions (sponsors and contract laboratories, where used) have warranted strict adherence to all applicable animal use regulations in the conduct of these studies. Although efforts were made to harmonize testing procedures and conditions, the local animal use regulations were always prioritized should any conflicts have arisen during the conduct of the study.

2.2. Experimental animals

All participating laboratories used purpose bred beagle dogs acquired from a vendor within their geographic region (North America or Europe). Some laboratories used only male dogs and other laboratories used both males and females. The source and sex of the dogs used by the various laboratories are summarized in Table 2.

Animals were either naïve at the study onset or they had been used previously during the conduct of safety pharmacology studies but were healthy and free of any residual test article at the start of the study. No animals were required to be euthanized in the context of this study. After an appropriate recovery period following surgery or washout period after receiving a drug, animals were subjected to a standard clinical pathology examination to evaluate their health status according to local procedures (typically including blood cell counts, serum electrolytes and biochemistry parameters indicative of kidney and liver function) and were qualified for use in further studies.

2.3. Telemetry instrumentation

Each participating laboratory used one of three commercially available implantable large animal telemetry systems; PhysioTel™ model D70-PCTP (Data Sciences International, St. Paul, MN), PhysioTel™ Digital model L21 (Data Sciences International, St. Paul, MN), or ITS model T27 (Konigsberg Instruments, Monrovia, CA). The system used by each laboratory is identified in Table 2.

Regardless of the telemetry system used, all dogs were instrumented to monitor aortic BP, LVP, the ECG, body temperature and activity. Note however that the temperature and activity endpoints were not evaluated in this study.

2.4. Surgery

Dogs were instrumented with the implantable telemetry system under general anesthesia using aseptic technique according to the procedures approved by the individual facility animal welfare oversight committee (IACUC/OB) and appropriate to the type of telemetry system being used. Postoperative analgesic, anti-inflammatory and antimicrobial drugs were administered according to approved institutional procedures. All animals were allowed to fully recover from surgery prior to study onset. In some cases, dogs were obtained from a pre-existing colony of instrumented animals that had previously been used for other safety pharmacology studies.

A general description of the surgical implantation procedure for each telemetry system is described in the following sections:

2.4.1. Konigsberg (ITS) T27

The sites that used Konigsberg (ITS) telemetry systems all used similar surgical procedures (Klumpp, Trautman, Markert, & Guth, 2006). The transducers of the T27 implant were calibrated and the unit was sterilized using a low pressure ethylene oxide process prior to implantation.

Anesthetized dogs were placed in a right lateral recumbency position and a skin incision was made between the fifth and sixth intercostal space. A small pocket was opened in the abdominal wall for implantation of the telemetry transmitter, battery housing, and induction switch coil. Both pressure transducer wires and the ECG leads extending from the transmitter were guided subcutaneously to the lateral incision. The initial incisions required for battery and transmitter placement were closed. A left thoracotomy was performed between the fifth and sixth intercostal space under intermittent positive pressure ventilation to expose the left ventricular apex for insertion of the pressure transducer, which was then secured with a purse string suture

A purse string suture was preplaced in the descending thoracic aorta, the aorta was temporarily occluded with a vascular clamp and the pressure transducer, which also served as the negative ECG electrode, was inserted through a stab incision. The transducer was secured in place by tightening the purse string suture and the vascular clamp was removed, thus restoring blood flow.

The lung was then inflated and the intercostal muscles were sutured closed and the air was removed from the pleural space. The chest wall was closed in layers and the skin was sutured.

After an appropriate recovery period, as defined by each participating laboratory, animals were returned to the colony of implanted dogs in group-housing conditions.

2.4.2. DSI PhysioTel™ D70-PCTP & PhysioTel™ digital L21

Four of the laboratories using DSI telemetry implants (D70-PCTP and L21) implanted the devices through an abdominal laparotomy approach, whereas one laboratory (D70-PCTP) used an intercostal thoracotomy approach.

The abdominal surgical implantation procedure for DSI implants was similar across all sites and device models. Some differences in surgical technique included: 1) placement of the implant body either within the peritoneal cavity or in a pocket between the peritoneum and the abdominal muscles, 2) since the L21 implant has a short external antenna, it was routed between the peritoneum and the abdominal muscle, whereas the D70-PCTP's antenna is internal, 3) some blood pressure sensor cannulae were inserted into a femoral artery while others were inserted into a branch of the mesenteric artery that perfuses the jejunum. However, in both cases, the artery or arterial branch was ligated and the sensor tip was advanced into the abdominal aorta. Note that all implants arrived pre-calibrated and sterile from the manufacturer.

The abdomen was approached via a longitudinal incision along the *linea alba*. The arterial sensor was routed either through the abdominal wall to the inguinal region where the femoral artery was exposed or directly into one of the arcuate branches of a jejunal mesenteric artery. Under intermittent positive pressure ventilation a dorso-ventral

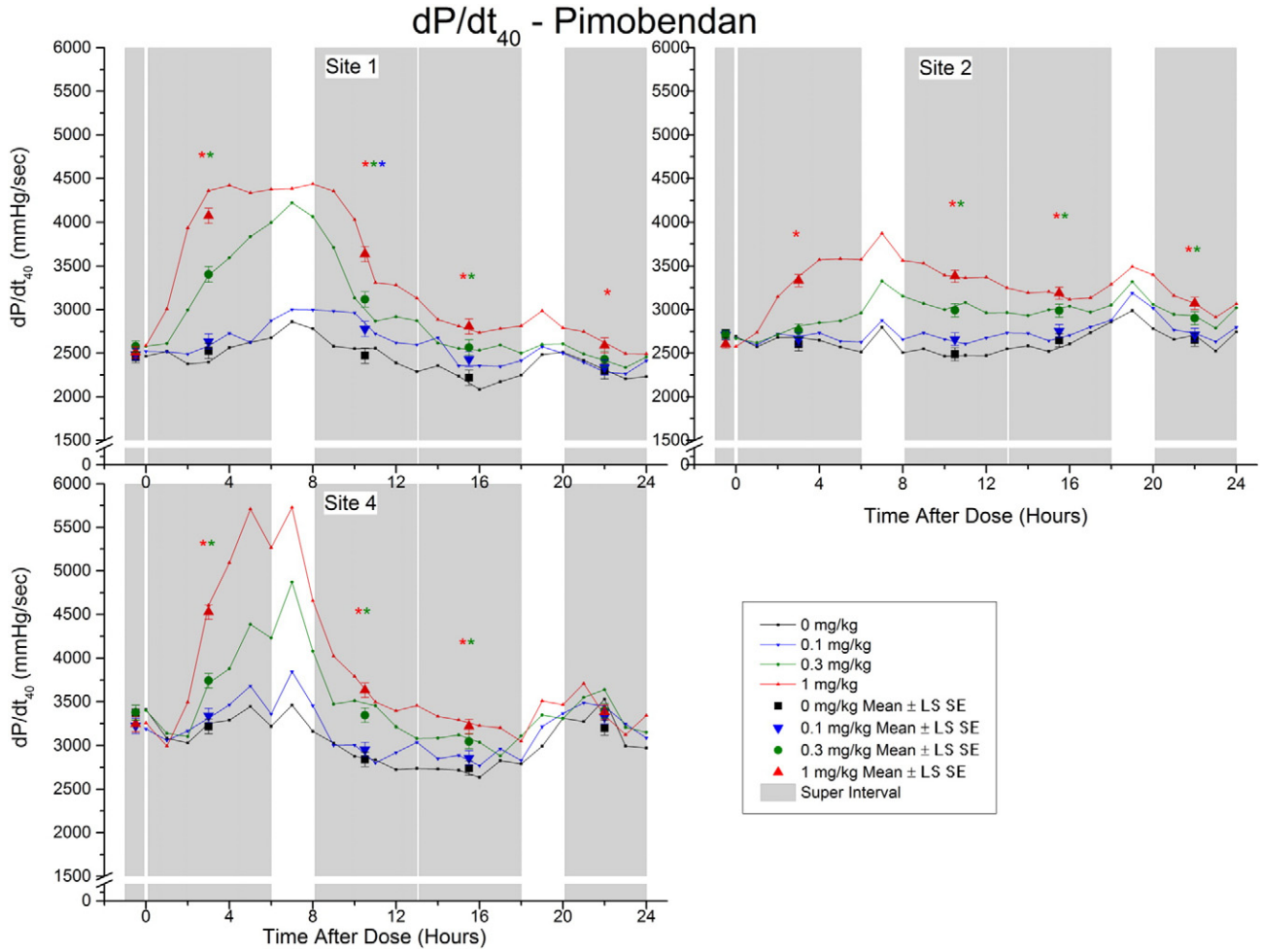

Fig. 4. The effect of pimobendan on LV dP/dt_{40} in conscious instrumented dogs at three different laboratories (sites 1, 2 and 4). Pimobendan (vehicle = □, 0.1 mg/kg = ∇, 0.3 mg/kg = ●, 1 mg/kg = Δ) was administered orally at time = 0. The small symbols represent the mean value from the previous 10-min while shaded areas represent super-intervals as defined in Table 3. Large symbols represent the mean ± SE within each super-interval.

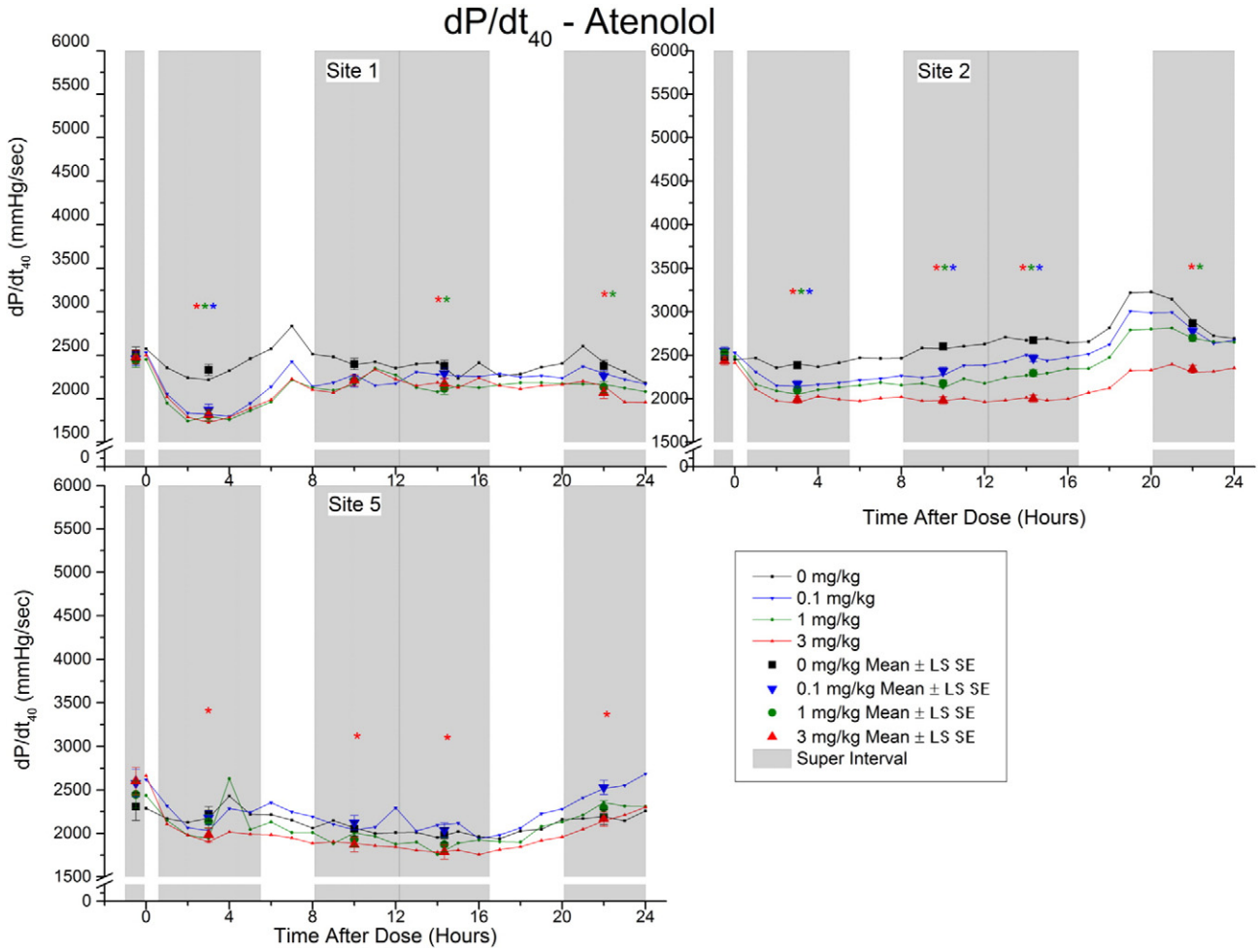

Fig. 5. The effect of atenolol on LV dP/dt_{40} in conscious instrumented dogs at three different laboratories (sites 1, 2 and 5). Atenolol (vehicle = □, 0.1 mg/kg = ▽, 0.3 mg/kg = ●, 1 mg/kg = Δ) was administered orally at time = 0. Small symbols represent the mean value from the previous 10-min while shaded areas represent super-intervals as defined in Table 3. Large symbols represent the mean ± SE within each super-interval.

incision was made in the diaphragm from the abdominal side, the pericardium was opened, and the left ventricular apex of the heart was exposed. A non-absorbable suture was placed around the suture aid near the tip of the pressure transmission catheter and tied. Another non-absorbable suture was placed around the left ventricular apex in a purse string pattern. The LV apex was perforated with a hypodermic needle, the perforation was dilated slightly with a straight Kelly forceps and the pressure sensing tip of the catheter was advanced into the LV chamber. After confirmation of a normal LVP signal, the purse string suture was tightened and tied. The tails of the suture that was previously tied around the catheter suture aid were then tied to the tails of the purse string to ensure the sensor could not back out of the ventricle.

The diaphragmatic incision was closed with braided absorbable suture in a continuous pattern. The lungs were gently inflated and the air was removed from the pleural space with a flexible catheter inserted between the continuous diaphragmatic sutures. The abdominal incision was closed in layers and the skin was closed with either continuous, subcuticular absorbable sutures, skin sutures or skin staples.

For the site that used the thoracotomy approach with the DSI device, the surgery was similar to that described previously (Henriques et al., 2010). Differences at other sites include the placement of one of the ECG electrodes on the left auricle of the heart and the second inside the thorax on a rib near the apex of the heart. Also, the systemic arterial pressure catheter was routed to the femoral artery and inserted up into the abdominal aorta.

2.5. Drugs tested

Four drugs were studied, each a known positive or negative inotropic drug in humans when given at clinical doses (Kato, 1997; Kullberg, Freeman, Biddlecome, Alousi, & Edleson, 1981; Qu et al., 2013; Thomas et al., 1992; Van Meel & Diederen, 1989; Ward, Brogden, Heel, Speight, & Avery, 1983). Not all test articles were studied in each laboratory (Guth et al., 2015). The doses and formulations used in this study are described in Table 1. Briefly, the test articles used included pimobendan (0.1, 0.3 & 1 mg/kg in PCCA Fixed Oil Suspension Vehicle ™), amrinone (0.5, 2 & 5 mg/kg dosed in gelatin capsules), itraconazole (3, 10 & 30 mg/kg in 0.5% (w/w) methocel E50 in water containing 0.01% (w/w) polysorbate 80 and 10 mM phosphate buffer (pH 6.80–7.20) and atenolol (0.3, 1 & 3 mg/kg in deionized water). Each drug included the appropriate vehicle control group.

2.6. Study design

Four different drug treatments were administered to each dog in the order prescribed by a randomly generated double Latin square design over four treatment days at each test site with an appropriate washout period between days (Guth et al., 2015). The washout period was a minimum of 72 h between treatment days. The double Latin square study design combines two identical 4 × 4 Latin squares (Sarazan et al., 2011).

The food provided was withdrawn approximately 2 h before dosing in the morning and reintroduced in the afternoon, which was well after the anticipated time to peak drug concentration (T_{max}) of the tested drug.

2.7. Pharmacokinetic concentration-response profiles and study exposure confirmation

A full pharmacokinetic profile of all four test compounds at the doses selected for use in this study was conducted by two of the participating companies. These data defined C_{max}/T_{max} as well as the time point at which all studies included a single plasma sample to confirm drug exposure in each animal, on each study day. The plasma samples were then frozen and, at study conclusion, bioanalytical measurements were performed.

2.8. Data collection and analysis

2.8.1. Raw data (signals)

Digital cardiac LVP, aortic BP and ECG signals were continuously acquired from at least 1 h prior to dosing through 24 h post dose on each study day. Sampling rates were ≥500 Hz for LVP and ECG signals and ≥250 Hz for BP signals which is adequate for the frequency content of each of these signal types (Sarazan, 2014). Digital raw data files were archived to electronic media and retained at each individual study site for future analysis as agreed upon within the HESI Cardiac Safety Technical Committee.

2.8.2. Derived data (parameters)

A variety of derived parameters was calculated by the data analysis software output from digital acquisition units at each study site and included: HR, diastolic aortic pressure, systolic aortic pressure, mean aortic pressure, pulse pressure, LV end-diastolic pressure, peak systolic LV pressure, $LVdP/dt_{max}$, $LVdP/dt_{min}$, $LVdP/dt_{40}$, Tau (or the left ventricular diastolic time constant) and the QA interval (or systolic time interval between the QRS complex and the onset of the aortic BP pulse). Although a large number of parameters were available, this manuscript is limited to cardiac function and to hemodynamic parameters that are directly or indirectly relevant to the evaluation of those cardiac contractility and lusitropy parameters described above.

2.8.3. Statistical analysis

Derived data was calculated for every cardiac cycle and the results were collapsed into 10-min mean values for analysis. These mean values were further averaged in order to construct the pre-specified large summary or super-intervals (Sivarajah et al., 2010) for each test article as shown in Table 3. The super-intervals used for each compound

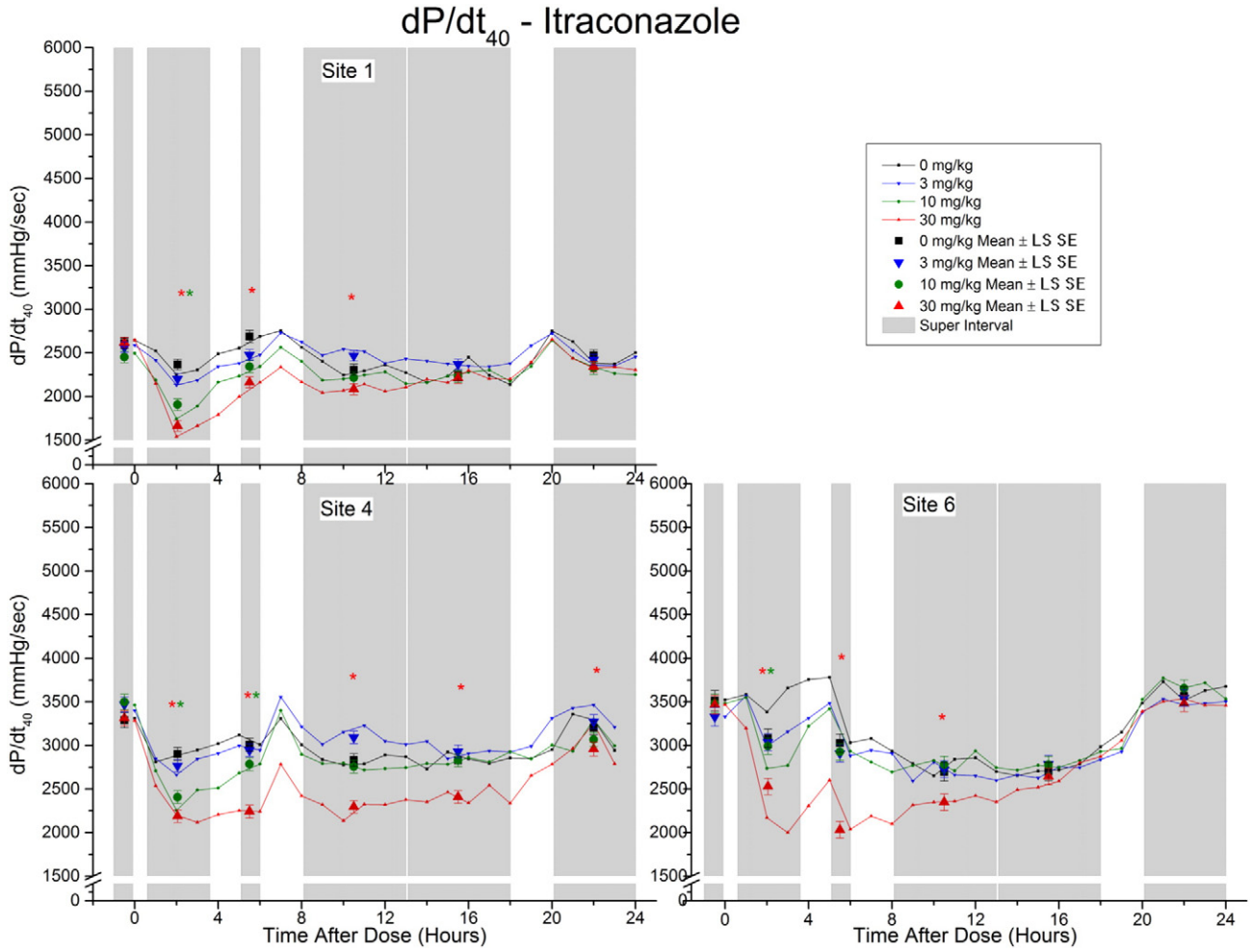

Fig. 6. The effect of itraconazole on LV dP/dt_{40} in conscious instrumented dogs at four different laboratories (sites 1, 4 and 6). Itraconazole (vehicle = □, 3 mg/kg = ∇, 10 mg/kg = ●, 30 mg/kg = Δ) was administered orally at time = 0. Small symbols represent the mean value from the previous 10-min while shaded areas represent super-intervals as defined in Table 3. Large symbols represent the mean ± SE within each super-interval.

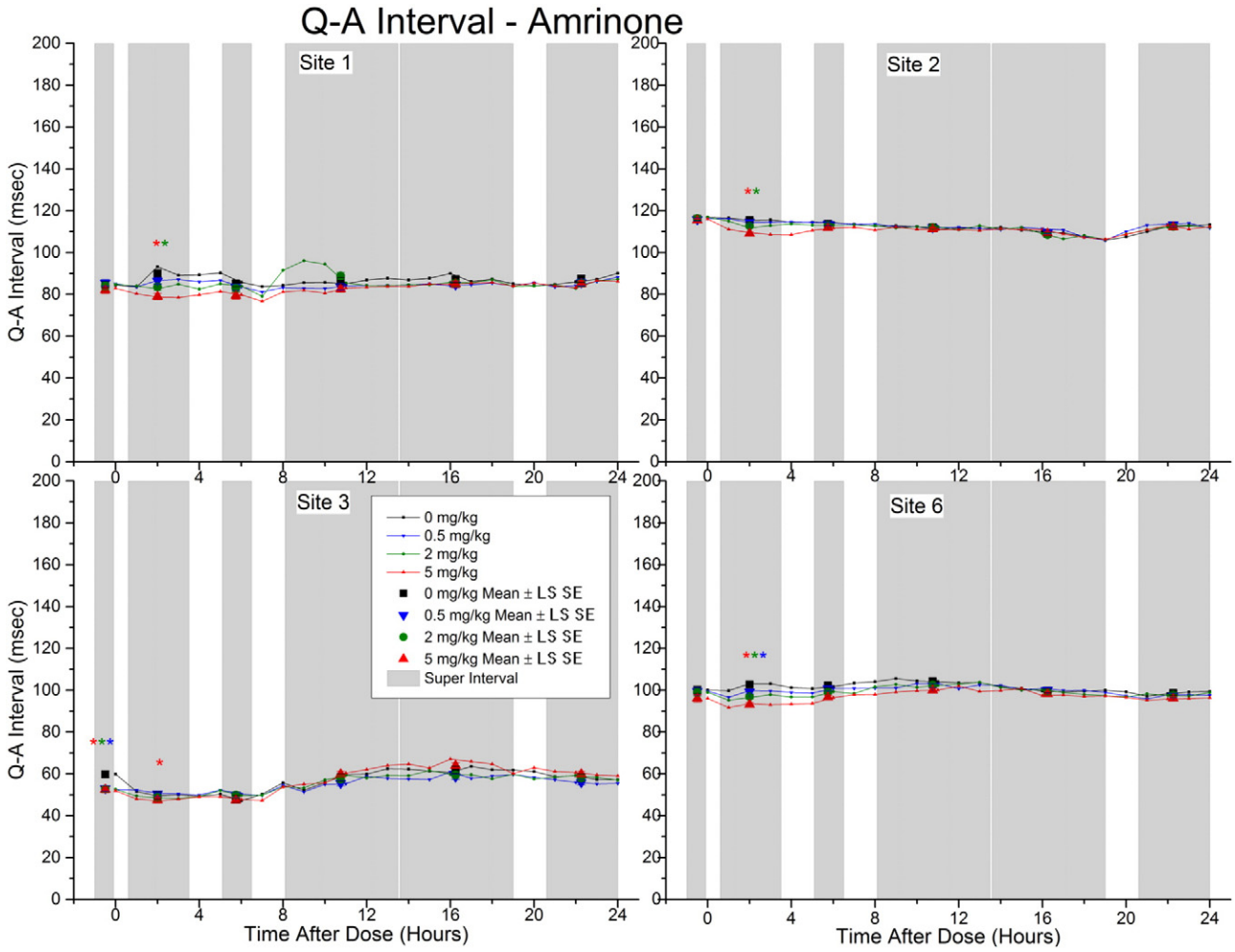

Fig. 7. The effect of amrinone on the QA interval in conscious instrumented dogs at five different laboratories (sites 1, 2, 3 and 6). Amrinone (vehicle = □, 0.5 mg/kg = ∇, 2 mg/kg = ●, 5 mg/kg = Δ) was administered orally at time = 0. Small symbols represent the mean value from the previous 10-min while shaded areas represent super-intervals as defined in Table 3. Large symbols represent the mean ± SE within each super-interval.

were defined by a data evaluation subteam prior to conduct of the statistical analysis. The selection of intervals was intended to limit variability associated with ambulatory dog cardiovascular assessments and avoids disturbances associated with dosing, changes in light cycle or at the time of blood sampling for drug exposure confirmation. Each compound was treated individually, selecting intervals from the average of $LVdP/dt_{max}$ across the laboratories that tested a given compound (Guth et al., 2015).

The effect of each test article on each of the cardiac parameters was evaluated using a repeated measures analysis of covariance (Chiang, Smith, Main, & Sarazan, 2004). Factors in the model included study day, animal, treatment group, time after dose, (pre-treatment) baseline, and the interaction of time after dose with each of the other factors. The within- and between-animal variability was modeled to follow a compound symmetric structure (Chiang et al., 2007). The effects of treatment and treatment by time interaction were tested by overall F-tests at the 0.01 significance level. Monotonicity of dose response for each super-interval was examined using sequential linear trend tests at the 0.05 significance level. Non-monotonic dose response profiles were investigated whenever no significant linear trends were detected but there was a significant overall F-test for either the treatment main effect or treatment by time interaction. The mean of each treatment group was compared to that of the vehicle group using Bonferroni adjusted *t*-tests at the 0.05 significance level.

3. Results

The results presented in this study are a continuation of the analysis of contractility data generated across multiple laboratories by Guth et al. (2015). Each of the laboratories used similar, but not identical, high fidelity telemetry recording technology in order to measure cardiovascular function in the conscious dog. Each laboratory was capable of producing similar experimental results on the inotropic and lusitropic effects of the drugs tested (Guth et al., 2015). In this manuscript, an overview of the effects of both positive and negative inotropic drugs were evaluated on the inotropic parameters $LVdP/dt_{40}$ and the QA interval (Fig. 1) as well as the myocardial relaxation parameters $LVdP/dt_{min}$ and Tau that were assessed across laboratories. In addition to assessment of drug effects on myocardial relaxation, results were interpreted as they relate to the plasma drug levels of the drugs tested.

Note that studies that evaluated the effects of amrinone on study parameters were conducted in 5 different laboratories; however, itraconazole was only evaluated in 4 different laboratories and both atenolol and pimobendan were each only tested in 3 laboratories

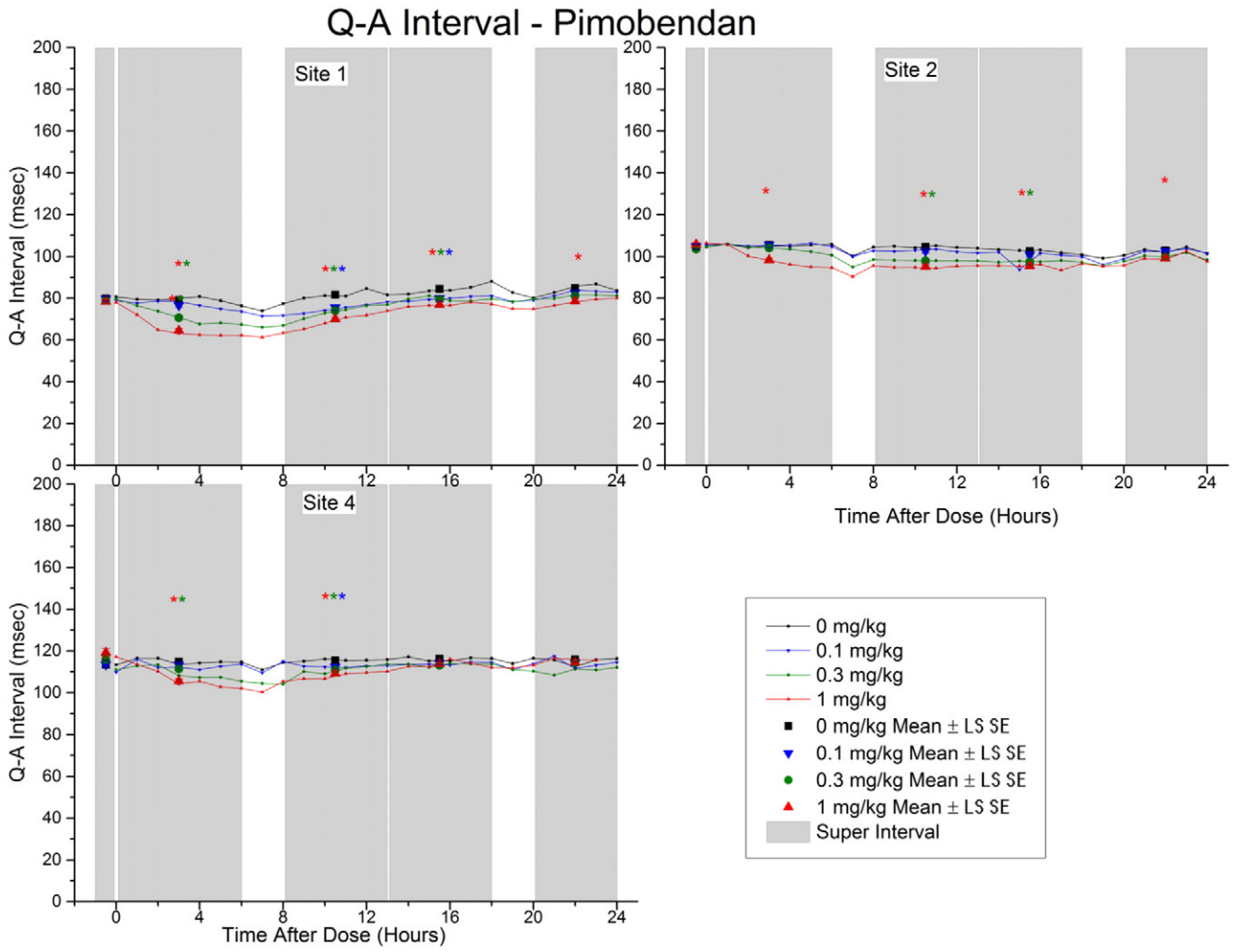

Fig. 8. The effect of pimobendan on the QA interval in conscious instrumented dogs at three different laboratories (sites 1, 2 and 4). Pimobendan (vehicle = □, 0.1 mg/kg = ∇, 0.3 mg/kg = ●, 1 mg/kg = Δ) was administered orally at time = 0. Small symbols represent the mean value from the previous 10-min while shaded areas represent super-intervals as defined in Table 3. Large symbols represent the mean ± SE within each super-interval.

(Table 1). The dose- and time-dependent effects of the four test compounds will also be discussed.

3.1. The performance of $LVdP/dt_{40}$ when compared to $LVdP/dt_{max}$ as a measure of LV contractility

The drug-induced changes produced by the four test drugs were evaluated by comparing their effects on $LVdP/dt_{max}$ vs. $LVdP/dt_{40}$ for each laboratory. It could be demonstrated that with this set of drugs the two parameters yielded nearly identical results in terms of the response observed. Although the magnitude of the effect observed was proportionally reduced for $LVdP/dt_{40}$ compared to $LVdP/dt_{max}$ the response profile was similar as was the duration of the effect (see Fig. 2). These parameters were found to be very similar in response to the tested drugs across all sites.

3.1.1. Positive inotropic drugs

3.1.1.1. Amrinone. As was observed with LV dP/dt_{max}, all laboratories that tested amrinone (a pyridine phosphodiesterase 3 (PDE3) inhibitor with vasodilator activity) showed a dose-dependent increase in LV dP/dt_{40} (Fig. 3). This response was primarily observed after the mid (2 mg/kg) dose but was statistically significant at the high (5 mg/kg) dose, although site 3 found an effect during the first 2 h post-dose (0.5–3.5 h super-interval) at the low dose (0.5 mg/kg). As with LV dP/dt_{max}, the duration of the response for drug-induced change in $LVdP/dt_{40}$ could be observed for the second super-interval at 3 sites.

Prior to drug administration, measure of $LVdP/dt_{40}$ was very consistent across test sites (Table 4). Values ranged from 2453 ± 40 at site 1 to 3637 ± 59 mmHg/s at site 4. These data are much more consistent than for $LVdP/dt_{max}$ which ranged from 2406 ± 58 mmHg/s at site 2 to 4894 ± 101 mmHg/s at site 4. However, regardless of the differences in baseline level, there appeared to be no differences in sensitivity (i.e., in the magnitude and statistical significance of the response to the drug across the sites) of the model to detect drug-induced increases in contractility for the drugs evaluated.

3.1.1.2. Pimobendan. Following administration of the mid (0.3 mg/kg) and high (1.0 mg/kg) doses of pimobendan, all 3 laboratories observed a dose-dependent, reversible increase in $LVdP/dt_{40}$, a response similar to that for $LVdP/dt_{max}$. The low (0.1 mg/kg) dose did not produce a significant effect on $LVdP/dt_{40}$ at any site (Fig. 4); however, the effect was statistically significant after administration of the high dose of pimobendan. The response of $LVdP/dt_{40}$ to pimobendan was very protracted and significant during the 20–24 h super-interval at site 1 of the study. The differences observed in baseline values between sites for $LVdP/dt_{40}$ had no apparent impact on the model to detect an effect of the drug.

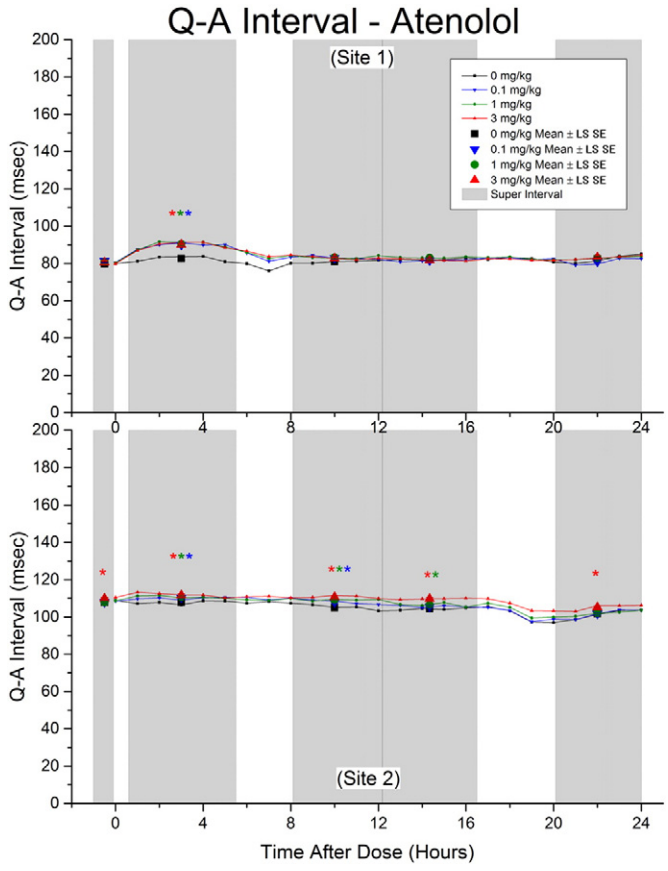

Fig. 9. The effect of atenolol on the QA interval in conscious instrumented dogs at two different laboratories (sites 1 and 2). Atenolol (vehicle = □, 0.1 mg/kg = ▽, 0.3 mg/kg = ●, 1 mg/kg = Δ) was administered orally at time = 0. Small symbols represent the mean value from the previous 10-min while shaded areas represent super-intervals as defined in Table 3. Large symbols represent the mean ± SE within each super-interval.

3.1.2. Negative inotropic drugs

3.1.2.1. Atenolol. While 3 laboratories assessed the effects of atenolol, only site 2 had a statistically significant dose-dependent decrease in $LVdP/dt_{40}$ with all 3 administered doses tested (0.3, 1 and 3 mg/kg) across all 4 defined super-intervals (Fig. 5). Site 5 only had a statistically significant decrease in $LVdP/dt_{40}$ at the highest dose tested, but it persisted across all super-intervals. Site 1 had only a statistically significant effect at the mid and high doses at the 0.5–5.5 h, 12.17–16.5 h and 20–24 h super-intervals; however, all post-dose mean values of $LVdP/dt_{40}$ were below the baseline values. When atenolol-mediated reductions in $LVdP/dt_{40}$ values were compared to those of $LVdP/dt_{max}$ they were consistent across sites (see Guth et al., 2015).

3.1.2.2. Itraconazole. When itraconazole was examined at 4 different study sites, all laboratories detected a dose-dependent, reversible decrease in $LVdP/dt_{40}$ (Fig. 6). The high dose was most consistent at significantly reducing $LVdP/dt_{40}$ while no laboratories detected an effect on $LVdP/dt_{40}$ with the low dose (3 mg/kg). A similar response profile was observed for $LVdP/dt_{max}$ (see Guth et al., 2015).

3.2. The QA interval as a measure of LV contractility in the conscious dog during inotropic interventions

The performance of the QA interval, as a calculated parameter responsive to changes in the inotropic state of the heart, was much more variable between the inotropic agents tested and between test sites, in contrast to $LVdP/dt_{max}$ and $LVdP/dt_{40}$. Baseline values for the QA interval varied between 53 ± 0.3 ms (at site 3) and 116 ± 1.3 ms (at site 2) (Table 4A & B).

3.2.1. Positive inotropic drugs (amrinone and pimobendan)

The positive inotropic effect of amrinone was detected as a modest, transient reduction in the QA interval by three of the four testing sites (Fig. 7). The magnitude of the decrease in the QA interval (indicative of an increase in myocardial contractility) was limited and restricted primarily to the first super-interval after dose administration. In contrast, the positive inotropic effect of pimobendan (Fig. 8) was consistently detected as a statistically significant decrease in the QA interval at the high dose across sites. A dose-dependent, reversible reduction in the QA interval could be demonstrated at multiple super-intervals across all sites, similar to that seen with $LVdP/dt_{40}$ and $LVdP/dt_{max}$.

3.2.2. Negative inotropic drugs (atenolol and itraconazole)

The negative inotropic effects of atenolol and itraconazole were detected using the QA interval as an index of contractility; however, the effect was of shorter duration, much more limited in its magnitude and no clear dose-dependency was demonstrated. It should be noted that data for the QA interval were available only from two sites for atenolol (Fig. 9). In comparison, a long-lasting and dose-dependent decrease in contractility was observed when either $LVdP/dt_{40}$ or $LVdP/dt_{max}$ was used. When the effect of itraconazole was examined, a clear negative inotropic effect was observed at all testing sites; however, a significant increase in the QA interval could only be observed between sites at the highest dose tested (Fig. 10). The dose-dependent response was much less apparent at all test sites for the QA interval when compared to that observed for either $LVdP/dt_{40}$ (Fig. 6) or $LVdP/dt_{max}$.

3.3. Response of the lusitropic parameter $LVdP/dt_{min}$ to positive and negative inotropic drug administration

Since cardiac lusitropy (relaxation) is an important component of ventricular systole, inotropy and lusitropy are intimately related. Relaxation is auxotonic meaning the contracting ventricular muscle shortens against an increasing load (Chemla, Coirault, Hébert, & Lecarpentier, 2000). Thus, drugs can have differential effects on relaxation-related processes that may be independent of effects on inotropic processes. Altered relaxation properties such as reduced or slowed rates of LV pressure reduction may provide insight into the potential pathophysiological effects exerted by the drug on the heart (Gillebert & Raes, 1994). The performance of the lusitropic parameters $LVdP/dt_{min}$ and Tau were evaluated. Since $LVdP/dt_{min}$ is sensitive to the inotropic state of the ventricle and dependent upon BP, HR, stroke volume and aortic pressure we examined drug effects on Tau (Raff & Glantz, 1981). Tau reflects cardiac relaxation and is not dependent upon hemodynamics, HR or any other measures of cardiac function.

3.3.1. Positive inotropic drugs

The calculated lusitropic parameter, $LVdP/dt_{min}$, was largely unaffected by all doses of amrinone tested, at all sites (Fig. 11). Any change in lusitropy that occurred resulted during the first super-interval but was only observed at one site where the effect on $LVdP/dt_{min}$ was modest and response manifest late when compared to the drug effect on the inotropic state. Similarly, there was no observed effect of pimobendan on $LVdP/dt_{min}$ (Fig. 12).

3.3.2. Negative inotropic drugs

When $LVdP/dt_{min}$ was examined, dose-dependent effects of atenolol were observed at two sites (sites 1 and 2), but not at the third, at which no clear effect at any dose could be demonstrated (Fig. 13). Similarly,

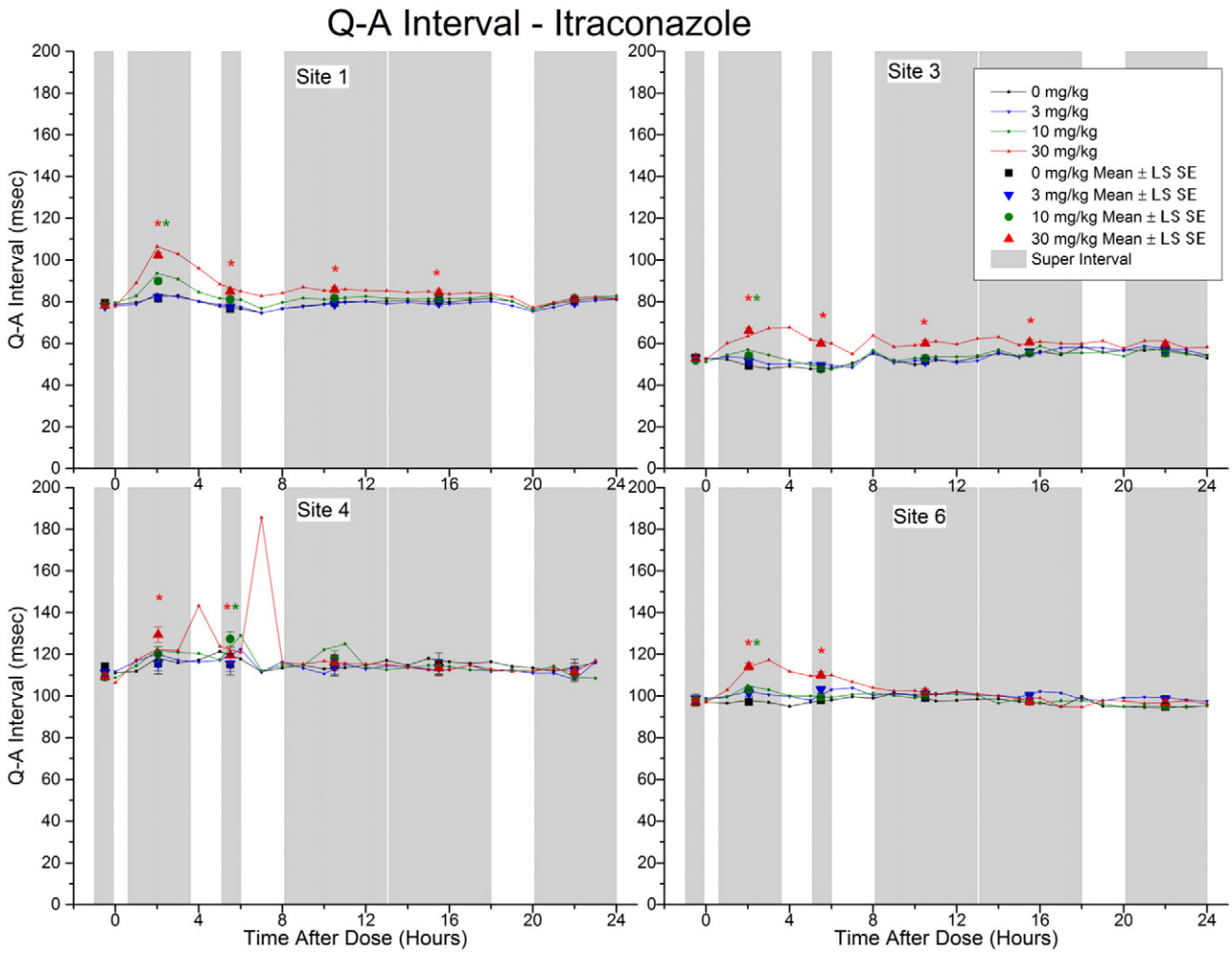

Fig. 10. The effect of Itraconazole on the QA interval in conscious instrumented dogs at four different laboratories (sites 1, 3, 4 and 6). Itraconazole (vehicle = □, 3 mg/kg = ∇, 10 mg/kg = ●, 30 mg/kg = Δ) was administered orally at time = 0. Small symbols represent the mean value from the previous 10-min while shaded areas represent super-intervals as defined in Table 3. Large symbols represent the mean ± SE within each super-interval.

when $LVdP/dt_{min}$, was used to assess the effects on itraconazole on myocardial relaxation, an inconstant and mostly transient effect was detected (Fig. 14).

3.4. Response of the lusitropic parameter Tau to positive and negative inotropic drugs

3.4.1. Positive inotropic drugs

Amrinone was found to produce a reduction in Tau in all of the 5 test sites where it was tested, but only a consistent statistically significant effect at all sites was seen with the high dose (5 mg/kg) (Fig. 15). The magnitude of the effect observed was variable between sites. A dose-dependent reduction of Tau was found by all three test sites for pimobendan (Fig. 16). Statistically significant effects were observed at the high dose since the magnitude and duration of the effect was variable at lower doses.

3.4.2. Negative inotropic drugs

Modest increases in Tau were found by all three test sites testing atenolol (Fig. 17) but no clear dose-dependent effect was identified. The high dose effect was statistically significant across sites. In studies with itraconazole, Tau was shown to increase with the high dose tested at 3 of the 4 sites where it was tested; however, the effect was transient and inconsistent across labs (Fig. 18).

3.5. Heart rate and blood pressure responses to the positive and negative inotropic drugs

Tables 4A and B describe the Least Square (LS) mean direct and derived baseline contractility and hemodynamic measures determined for each group of study animals at each study site (1–6). The values in the Tables show the average of the 4 dose level baseline LSMean values with corresponding standard error (SE) of the pre-dose (i.e., 0 h time internal) measures of recorded parameters for the first super-interval in each of Figs. 2–18.

The LSMean BP and HR measures determined for each group of study animals at each study site (1–6) are shown in Table 5. These values define the hemodynamic changes resulting pre-dose (0 h time interval) and 0.5, 2 and 5 h after dosing, which bracket the maximal plasma concentration (C_{max}) for both positive and negative inotropic drugs tested at the super-intervals (as outlined in Table 3) associated with C_{max}. For both positive (amrinone and pimobendan) and negative inotropic (atenolol and itraconazole) drugs the doses administered did not produce significant effects on heart rate. Similarly, no significant effects were observed on blood pressure or

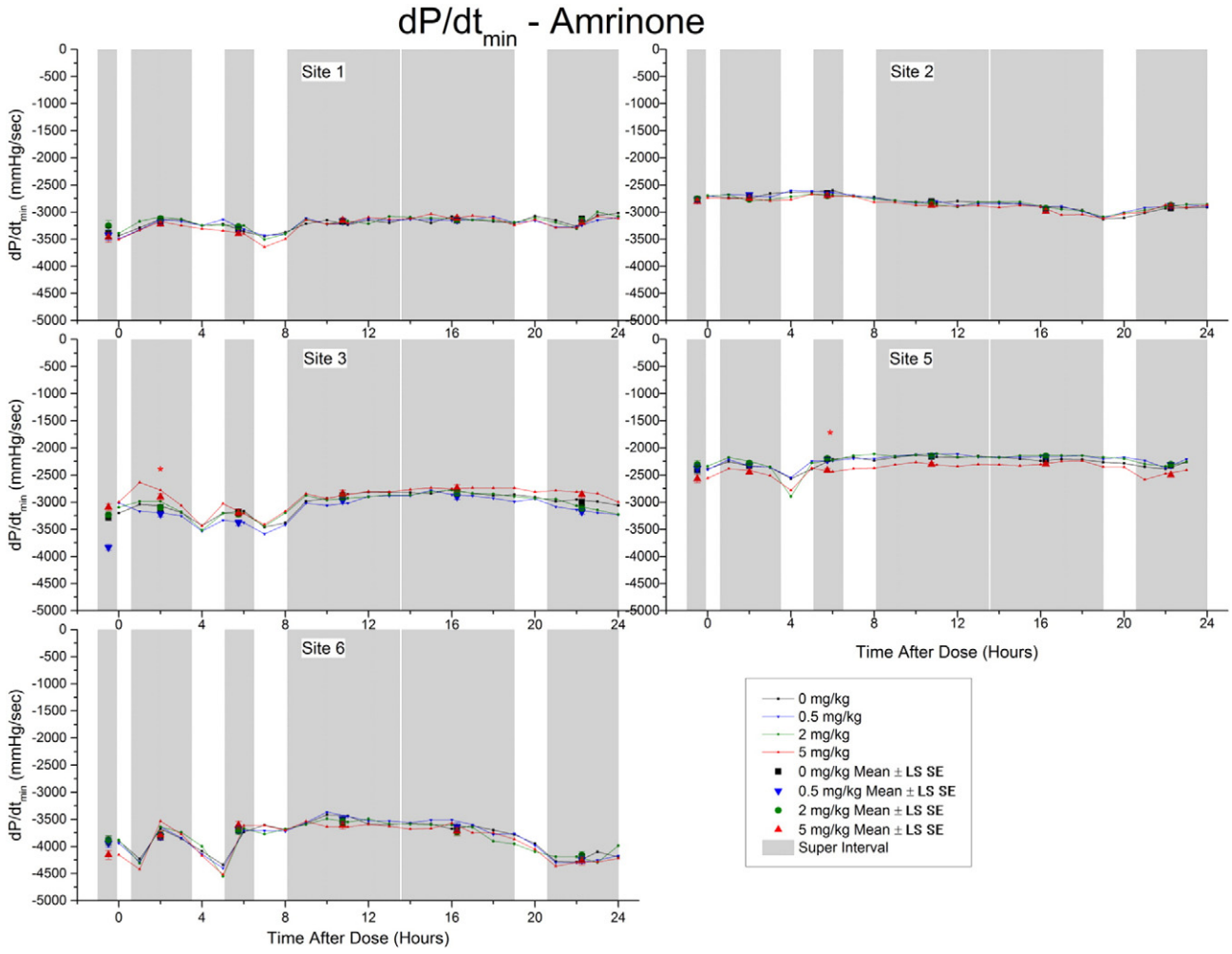

Fig. 11. The effect of amrinone on LV dP/dt$_{min}$ in conscious instrumented dogs at five different laboratories (sites 1, 2, 3, 5 and 6). Amrinone (vehicle = □, 0.5 mg/kg = ∇, 2 mg/kg = ●, 5 mg/kg = Δ) was administered orally at time = 0. Small symbols represent the mean value from the previous 10-min while shaded areas represent super-intervals as defined in Table 3. Large symbols represent the mean ± SE within each super-interval.

LVEDP suggesting no drug-mediated effects, at the doses given, on pre-load or afterload conditions in the animals. It should be noted, however, that while LVEDP did not change this is no indicative of changes in cardiac preload in the study since preload depends upon relaxation and the developed transmural pressure gradient (Berne & Levy, 1977). Similarly, the recorded arterial BP alone is not an index of cardiac afterload, rather this depends upon LV end diastolic volume (EDV) and ventricular wall thickness (Berne & Levy, 1977). The lack of drug effects are important to recognize in drug safety, especially in light of development of drugs for LV systolic heart failure such as omecamtiv mecarbil. This drug is a cardiac specific myosin (or molecular motor) activator that improved systolic function by increasing ejection time without increasing myocardial energy demands or LVdP/dt (Liu, Dorhout, van der Meer, Teerlink, & Voors, 2016).

3.6. Plasma drug levels

3.6.1. Pharmacokinetic concentration-response profile

The pharmacokinetic profiles of the four test drugs used in this study were characterized in dedicated pharmacokinetic studies prior to the conduct of the primary study in order to determine the concentration-time relationship of each compound after each dose administered orally to Beagle dogs. This information was used to define the time of blood withdrawal for exposure confirmation in the primary studies. It also served to define the super-intervals used for statistical evaluation of the data (see section 2.8.3 and Table 3). The summary data from these 4 pharmacokinetic studies are briefly summarized below.

3.6.1.1. Amrinone. When amrinone was administered orally at doses of 0.5, 2 or 5 mg/kg it resulted in peak plasma concentrations (C_{max}) of 269 ± 64, 743 ± 257 and 2652 ± 1096 ng/mL and AUC (ng·h/mL) values of 1247 ± 152, 4448 ± 1256 and 13,795 ± 4264, respectively. The amount of time that the drug was present at the maximum plasma concentration (T_{max}) was similar for each concentration and ranged between 1.7 and 2.7 h post-dose.

3.6.1.2. Pimobendan. When pimobendan was administered orally at doses of 0.1, 0.3 and 1.0 mg/kg, the resulting C_{max} levels were 1.9 ± 0.6, 7.3 ± 2.7 and 22.9 ± 13.5 ng/mL with AUC values of 4.4 ± 1.6, 22.5 ± 10.4 and 89.7 ± 17.8 ng·h/mL, respectively. These occurred at T_{max} values of 3.3, 3.2 and 2.3 h post-dose, respectively.

3.6.1.3. Atenolol. When atenolol was administered orally at doses of 0.3, 1 and 3 mg/kg, the C_{max} values were 174 ± 53, 538 ± 92 and 1718 ±

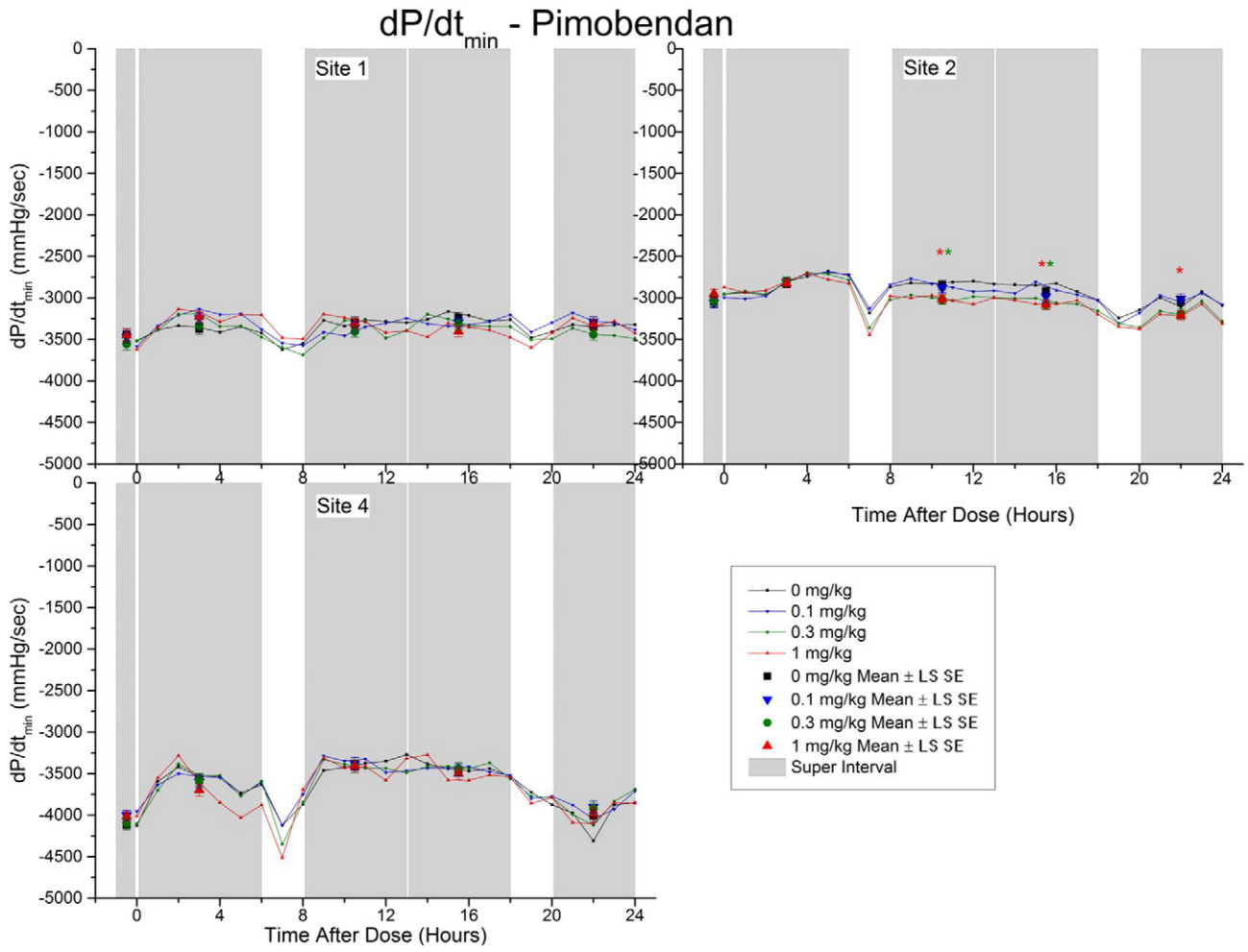

Fig. 12. The effect of pimobendan on LV dP/dt_{min} in conscious instrumented dogs at three different laboratories (sites 1, 2 and 4). Pimobendan (vehicle = □, 0.1 mg/kg = ∇, 0.3 mg/kg = ●, 1 mg/kg = Δ) was administered orally at time = 0. Small symbols represent the mean value from the previous 10-min while shaded areas represent super-intervals as defined in Table 3. Large symbols represent the mean ± SE within each super-interval.

364 ng/mL, with corresponding AUC values of 997 ± 143, 3333 ± 10.4 and 9425 ± 1026 ng·h/mL, respectively. These occurred at T_{max} values of between 1.3 and 1.5 h post-dose.

3.6.1.4. Itraconazole. When doses of itraconazole (3, 10 and 30 mg/kg) were given orally, these were associated with C_{max} values of 373 ± 92, 1253 ± 222 and 2127 ± 838 ng/mL and AUC values of 6748 ± 1605, 23,083 ± 5184 and 47,450 ± 13,794 ng·h/mL, respectively. The corresponding T_{max} values were between 2.3 and 2.7 h post-dose.

3.6.2. Contractility assessment study plasma drug concentrations

For a majority of studies, plasma samples were withdrawn at a single time point in order to confirm that animals in the contractility assessment study were administered drug doses that provided exposure multiples at the anticipated range based on the preliminary pharmacokinetic studies that were conducted. Table 5 provides a summary confirmation of the anticipated exposure levels from the contractility assessment study. There was a good exposure consistency across laboratories (see Guth et al., 2015 for individual site values). Note that the exposure data described in Table 5 do not represent the maximal drug concentrations that were determined in the preliminary pharmacokinetic studies since the timing of blood withdrawal was modified to follow T_{max} in order to avoid confounding artifacts in the cardiac function and hemodynamic parameters at the time of maximal plasma concentrations (Table 6).

4. Discussion

In this study, two positive inotropic and two negative inotropic drugs were administered orally to dogs chronically instrumented with telemetry devices in order to evaluate the effects of these drugs on both inotropic (dP/dt_{40} and QA) and lusitropic (dP/dt_{min} and Tau) measures of LV cardiac function. It is long recognized that drugs can produce effects on the contractile state of the myocardium and, when they occur, are important factors to be considered in the risk-benefit relationship during the development of any new drug. As has been discussed previously (Sarazan, Kroehle, & Main, 2012), both increases and decreases in the inotropic state of the heart have potential life-threatening consequences, depending upon several conditions such as the patient population and underlying disease. For that reason, the testing of new drug candidates for possible effects on the contractile state of the heart should be conducted routinely even if this is not explicitly outlined in current regulatory guidance documents.

One explanation as to why drug effects on contractility are not routinely measured may relate to the perception that a reliable approach for this assessment was not generally accessible to pharmaceutical

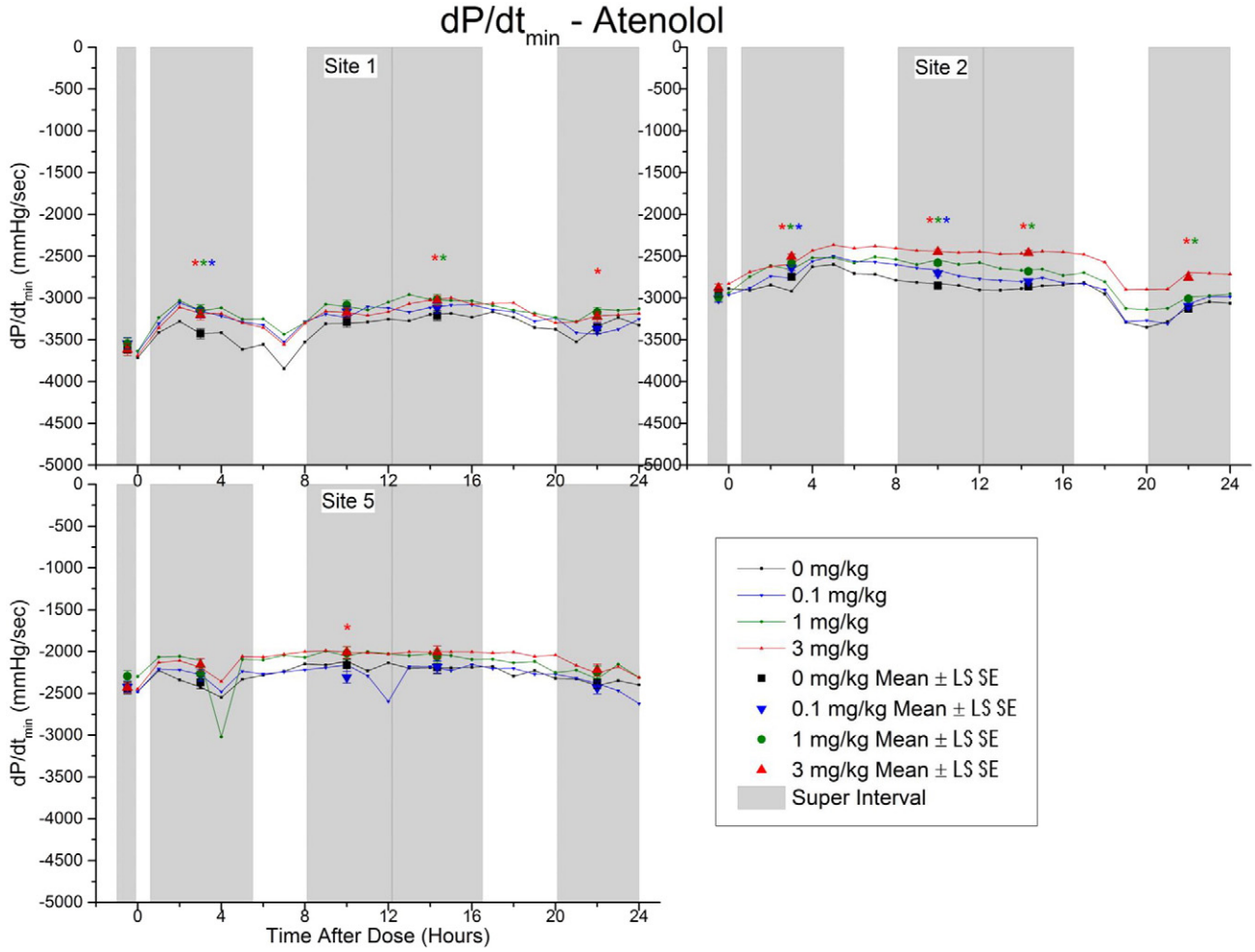

Fig. 13. The effect of atenolol on LV dP/dt_{min} in conscious instrumented dogs at three different laboratories (sites 1, 2 and 5). Atenolol (vehicle = □, 0.1 mg/kg = ∇, 0.3 mg/kg = ●, 1 mg/kg = Δ) was administered orally at time = 0. Small symbols represent the mean value from the previous 10-min while shaded areas represent super-intervals as defined in Table 3. Large symbols represent the mean ± SE within each super-interval.

companies or contract research organizations (CROs). Therefore, ILSI/HESI supported a consortium effort to demonstrate the robustness of the measurement of myocardial contractility in the conscious Beagle dog, initially using the maximal rate of left-ventricular pressure development, $LVdP/dt_{max}$ (Guth et al., 2015). The selection of $LVdP/dt_{max}$ as the parameter of choice was based on the experience of the investigators involved in the consortium and the fact that is has historically been used, and remains, the most common index of ventricular performance. However, $LVdP/dt_{max}$ is of limited potential as an independent measure of contractility since it is affected by changes in either the preload or afterload of the left ventricle (Gleason & Braunwald, 1962; Mason et al., 1971). In our previous publication (Guth et al., 2015), it was determined that the drugs and doses tested did not have a profound effect on either the preload (as assessed using the left ventricular end-diastolic pressure) or the afterload (as assessed using the diastolic aortic or arterial pressure), such that the use of $LVdP/dt_{max}$ as an index of contractility was unaffected. Nevertheless, in cases where the loading conditions of the left ventricle are more markedly affected by the NCE, the use of $LVdP/dt_{40}$ could offer some advantages. For that reason, $LVdP/dt_{40}$ was evaluated in the present paper using the same raw data set as was used for determination of $LVdP/dt_{max}$. Furthermore, we recognize that it is not always possible to have access to high fidelity recording equipment or access to more complex surgical procedures for measurement of left ventricular pressure, such that an additional index of contractility, independent of LVP measurement, may be desirable. The QA interval has been presented as such an option, as it requires only an ECG and an arterial blood pressure signal for measurement (Adeyemi et al., 2009; Norton et al., 2009). This paper provides, for the first time, an evaluation of the QA interval and $LVdP/dt_{40}$ in the conscious Beagle dog, both of which may be compared to $LVdP/dt_{max}$ from the previous work (Guth et al., 2015). On the basis of these data, $LVdP/dt_{40}$ was similar to $LVdP/dt_{max}$ in terms of its performance as an index of contractility. In contrast, while the simplicity of the measurement of the QA interval was found desirable, it was more variable in its response to inotropic changes across testing sites and, in general, appeared less sensitive to the drugs tested, particularly at the lower dose ranges of the drugs evaluated.

4.1. Load dependency of $LVdP/dt_{max}$

A possible explanation for the comparable performance of $LVdP/dt_{40}$ to that of $LVdP/dt_{max}$ may be that in this study the drugs and doses used were not associated with large changes in either LV end-diastolic pressure or arterial BP (Guth et al., 2015). As such, the potential advantage of $LVdP/dt_{40}$ due to its lesser dependence on ventricular loading conditions may not have been entirely manifest. Perhaps if higher doses of drugs in this study had been tested, or drugs had been tested that not only affect the contractile state of

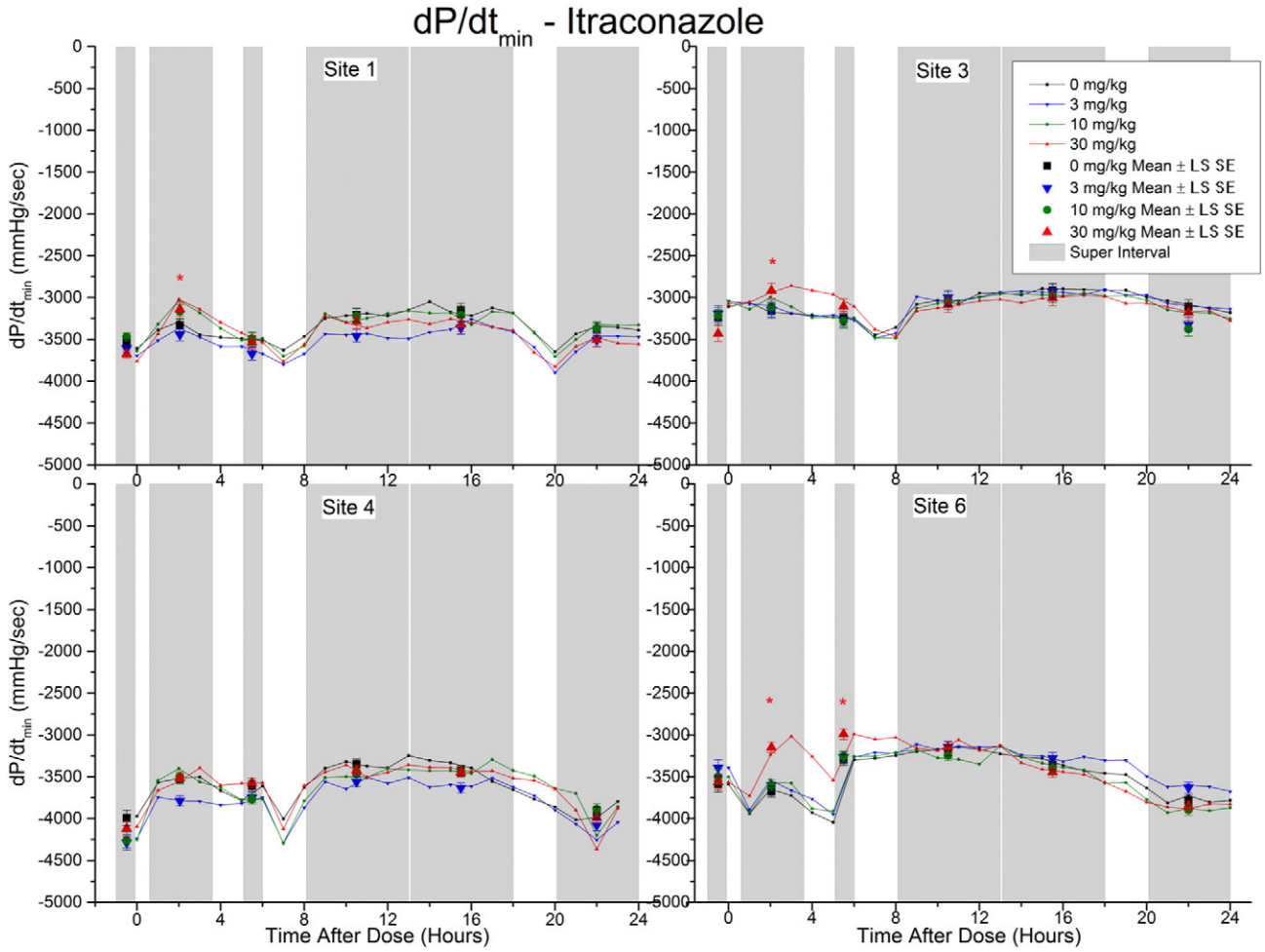

Fig. 14. Effect of itraconazole on LV dP/dt_{min} in conscious instrumented dogs at four different laboratories (sites 1, 3, 4 and 6). Itraconazole (vehicle = □, 3 mg/kg = ∇, 10 mg/kg = ●, 30 mg/kg = Δ) was administered orally at time = 0. Small symbols represent the mean value from the previous 10-min while shaded areas represent super-intervals as defined in Table 3. Large symbols represent the mean ± SE within each super-interval.

the heart but also have effects on the peripheral vascular system, $LVdP/dt_{40}$ may have demonstrated firm advantages for use compared to $LVdP/dt_{max}$. Alternatively, studies have shown that the magnitude of the load sensitivity of $LVdP/dt_{max}$ has been demonstrated repeatedly to be modest within a normal cardiac physiological pressure range (Gleason & Braunwald, 1962; Mason et al., 1971; Noda, Cheng, De Tombe, & Little, 1993) and that changes in both preload and afterload (or heart rate associated with tachycardia or bradycardia) need to be profound before an impact on $LVdP/dt_{max}$ can be demonstrated (Markert et al., 2007; Siegel & Sonnenblick, 1963; Zimpfer & Vatner, 1981). This would suggest, using the data derived from this study, that there appears little inherent advantage in the use of $LVdP/dt_{40}$ vs. $LVdP/dt_{max}$ and that both indices would qualify for use in this type of study, a conclusion also reached by Mahler et al. ~40 years ago (Mahler, Ross, O'Rourke, & Covell, 1975).

4.2. Modest performance of the QA interval as an index of contractility

The clear advantage of the use of the QA interval as an index of cardiac contractility is that it can be measured without the need for a high fidelity LVP signal (Jackson, 1974). Although current recording systems are capable of measuring LVP in chronically instrumented small rodents such as the rat or mouse (Tang et al., 2016), the most commonly used cardiovascular based measurement system does not usually include an implanted left ventricular catheter. The use of the ECG and arterial BP signals derived from such a basic system could be used to provide a contractility index (Cambridge & Whiting, 1986). The interest in qualifying the QA interval as a contractility index, even in larger animals such as the dog, can be understood from the perspective of the relative ease in instrumenting animals without a left ventricular catheter or micromanometer. Surgical implantation does require special training and is performed best by individuals having adequate expertise and practice. Furthermore, there have been concerns raised with regard to the catheter or micromanometer itself and its placement within the left ventricle. If the catheter is too long or a micromanometer advanced too far into the ventricular cavity they may press against the ventricular wall and could be the source of ectopic beats. Thus, an easy to use alternative for the assessment of cardiac contractility in drug safety is long-awaited.

The results of this paper, however, do not provide data supportive of the use of the QA interval as a sensitive surrogate index of cardiac contractility, certainly not in comparison to either $LVdP/dt_{max}$ or $LVdP/dt_{40}$. While contractile effects could be demonstrated they were, for the drugs tested at most sites, primarily restricted to the highest dose tested suggesting that the QA interval is less sensitive and therefore not sufficiently robust or capable of detecting subtle changes in ventricular contractility. This limitation of the use of the QA interval has not been a focus in previous

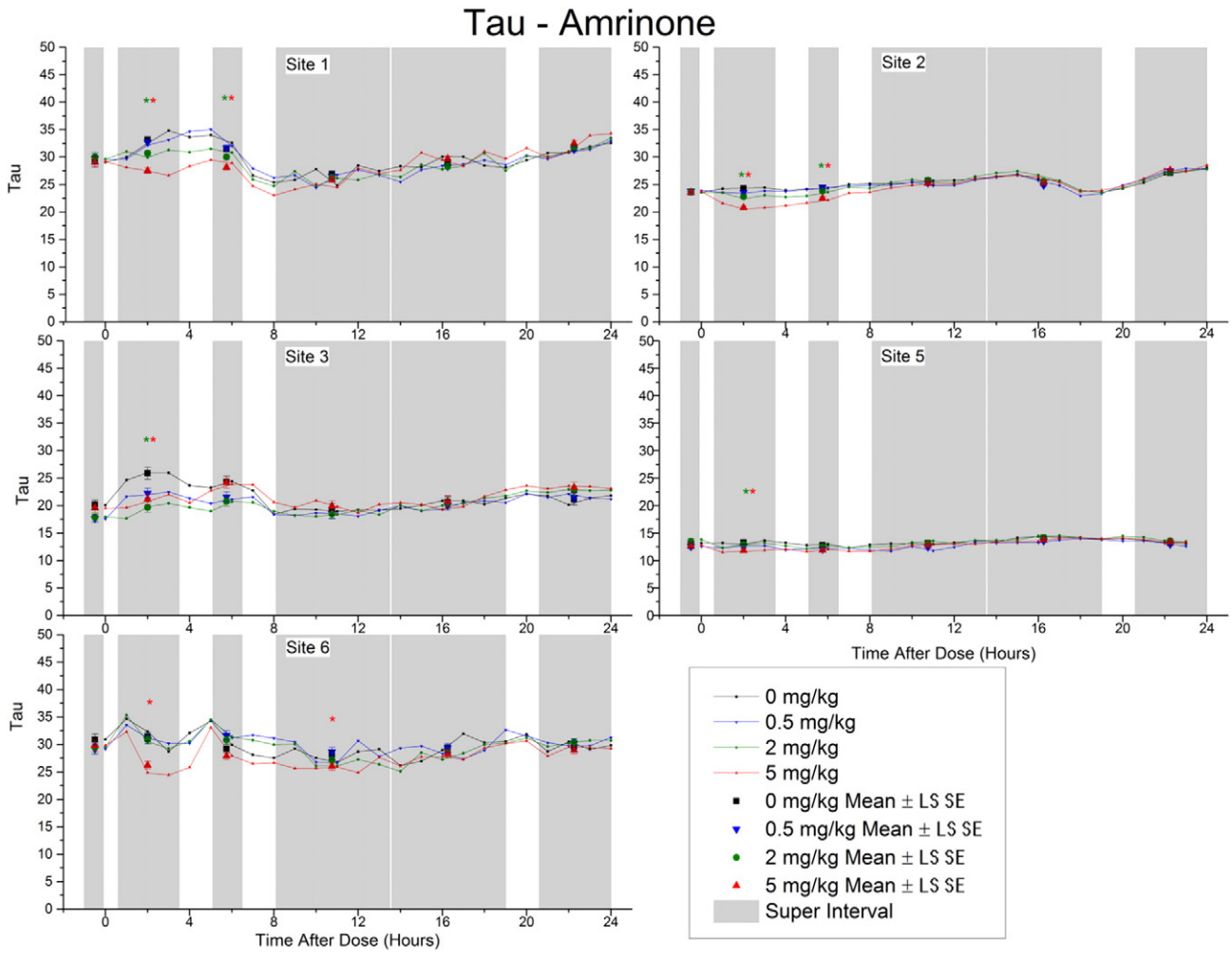

Fig. 15. The effect of amrinone on Tau in conscious instrumented dogs at five different laboratories (sites 1, 2, 3, 5 and 6). Amrinone (vehicle = □, 0.5 mg/kg = ▽, 2 mg/kg = ●, 5 mg/kg = △) was administered orally at time = 0. Small symbols represent the mean value from the previous 10-min while shaded areas represent super-intervals as defined in Table 3. Large symbols represent the mean ± SE within each super-interval.

critiques of the parameter. Rather, it has been acknowledged that the QA interval is subject to multiple effects that do not relate to the inotropic state of the heart but rather relate to several factors that include: transit times of aortic BP pulse responses and detection; required modification of ECG lead placement (Sgoifo method) to improve recorded signals (Adeyemi et al., 2009); anticipated changes in QA are small and require high sampling rates (i.e., 1000 Hz) (Adeyemi et al., 2009) and optimal placement of the blood pressure catheter tip (Cambridge & Whiting, 1986). Most importantly, the onset (A) of the upstroke of the aortic blood pressure pulse wave, which is indicative of the opening of the aortic valve (and onset of LV ejection) is dependent upon the diastolic arterial blood pressure (Cambridge & Whiting, 1986). In the context of the present study, however, this should not have been an important influence on the QA interval, as changes in arterial BP with the drugs and doses used were limited. In these studies, the parameter appeared to be less sensitive to changes in contractile function, suggesting that it could be useful but only in detecting comparatively large drug-induced changes in the inotropic state and, thus, more subtle effects may not be detected. Alternatively, such an uncomplicated measure may have utility in discovery screening assays, early frontloading cardiovascular safety pharmacology or toxicology studies since it can be determined in many rodent and non-rodent species where LV pressure measures are often not collected.

4.3. Drug-induced effects on the lusitropic state of the heart

It must be recognized that the primary goal of the ILSI/HESI consortium was not to evaluate the lusitropic effects of the drugs selected, doses tested and parameters (dP/dt_{min} and Tau) that may be used to detect such changes in cardiac function. Nevertheless, the clinical importance of drug-induced effects on the lusitropic state of the heart has developed (Chemla et al., 2000; Gillebert & Raes, 1994) such that the detection of lusitropic effects, when they occur, should also be integrated into the risk-benefit assessment of any new drug in development. With the unique data set generated with this consortium project, it was therefore decided to use these data for the calculation of the lusitropic parameters and to assess their changes in the presence of the positive and negative inotropic agents tested. Although, based upon the pharmacological nature of the drugs selected, there might be some anticipated effect on the lusitropic state of the heart with the agents used, it should be emphasized that their lusitropic effect was not the basis of their selection such that the effects observed may not

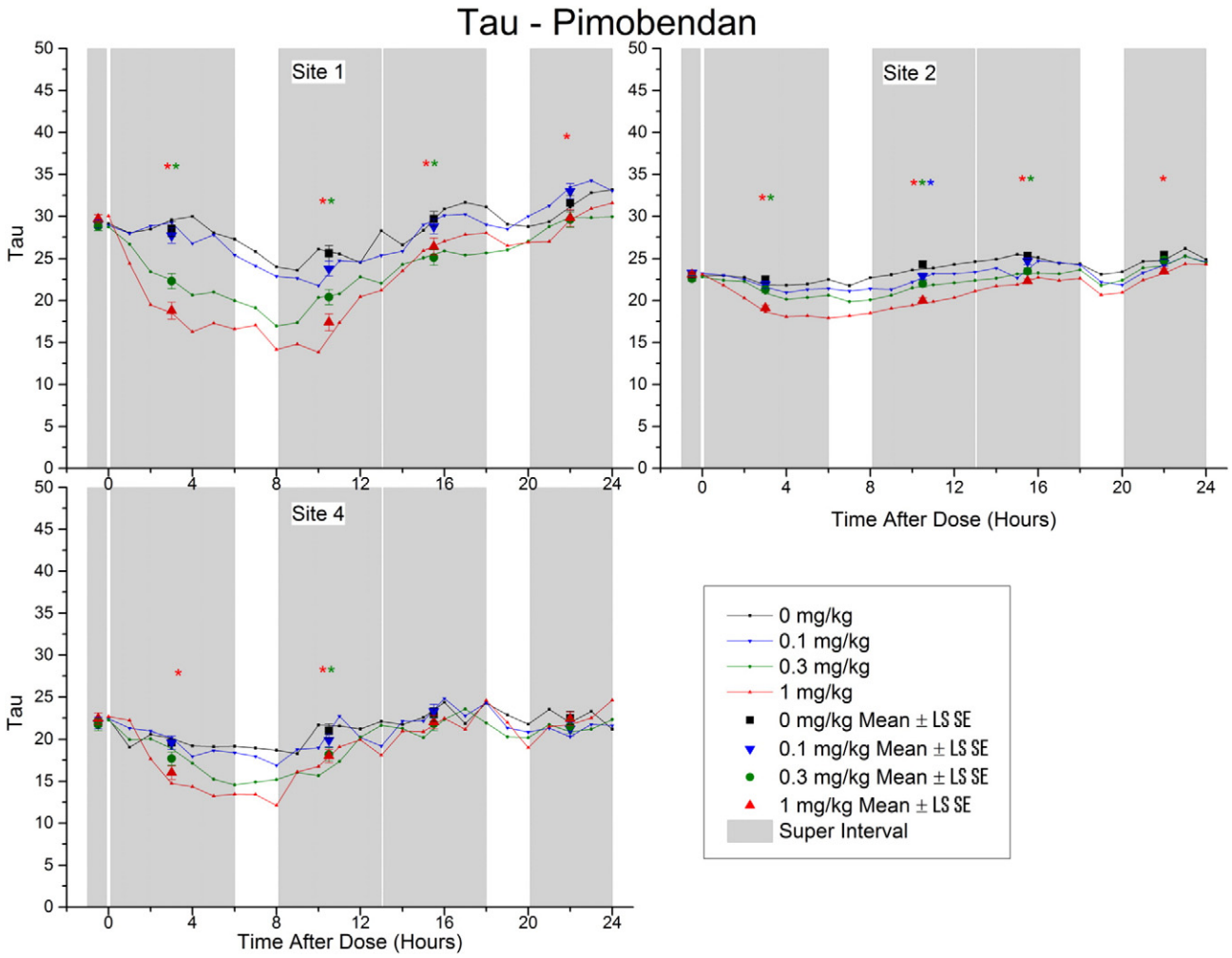

Fig. 16. The effect of pimobendan on Tau in conscious instrumented dogs at three different laboratories (sites 1, 2 and 4). Pimobendan (vehicle = □, 0.1 mg/kg = ∇, 0.3 mg/kg = ●, 1 mg/kg = Δ) was administered orally at time = 0. Small symbols represent the mean value from the previous 10-min while shaded areas represent super-intervals as defined in Table 3. Large symbols represent the mean ± SE within each super-interval.

reflect the failure of the measurements, per se, but rather on the drugs tested.

The two lusitropic parameters selected were $LVdP/dt_{min}$ and Tau. $LVdP/dt_{min}$ is the maximal or peak rate of pressure decrease in the left ventricle, whereas Tau is the LV diastolic time constant for isovolumetric left pressure decay (Raff & Glantz, 1981). An important distinction should be made, however, that the peak rate of LV relaxation is not the same measure as relaxation time. While the calculation of $LVdP/dt_{min}$ measures the peak negative derivative of LV pressure, the calculation of Tau may be sensitive to prolongation of relaxation time that may occur at either the beginning or end of isovolumic diastole (Raff & Glantz, 1981; Varma, Owen, Smucker, & Feldman, 1989). It is also likely that Tau may be more influenced by changes in heart rate than $LVdP/dt_{min}$. (Frederiksen, Weiss, & Weisfeldt, 1978; Weiss, Frederiksen, & Weisfeldt, 1976). This is evident in the responses to atenolol, where Tau is increased slightly in control animals that also showed modest decreases in heart rate from baseline conditions. However, neither $LVdP/dt_{min}$ nor Tau were shown to markedly change at the doses of the drugs tested in this study. A more definitive assessment of the value of these parameters may need to await evaluation of drugs known to have clinically relevant effects on the lusitropic state of the heart. For example, ONO-4232 is a selective agonist for the EP4 subtype of the prostaglandin E2 receptor and has dual left ventricular lusitropic and venodilatory activity and is currently in clinical development for use in patients with acutely decompensated heart failure (Ward et al., 2016).

4.4. Limitations of the study

The drugs that were used in this study were evaluated at doses that would result in plasma drug levels in dogs that are low multiples of clinically relevant therapeutic plasma concentrations. However, while the pharmacological activity of each drug at the doses evaluated is known to either increase or decrease cardiac contractility it is well established that these drugs are not pure modulators of inotropic function. Rather, these drugs produce effects on preload and afterload parameters not specifically evaluated by simple measurement of LVEDP or blood pressure, respectively, which could taint the study readout regarding drug effects on inotropy. The additional critical evaluation of all preload and afterload parameters that affect cardiac inotropy would assist in substantiating the use of $LVdP/dt_{40}$ as a measure of contractility.

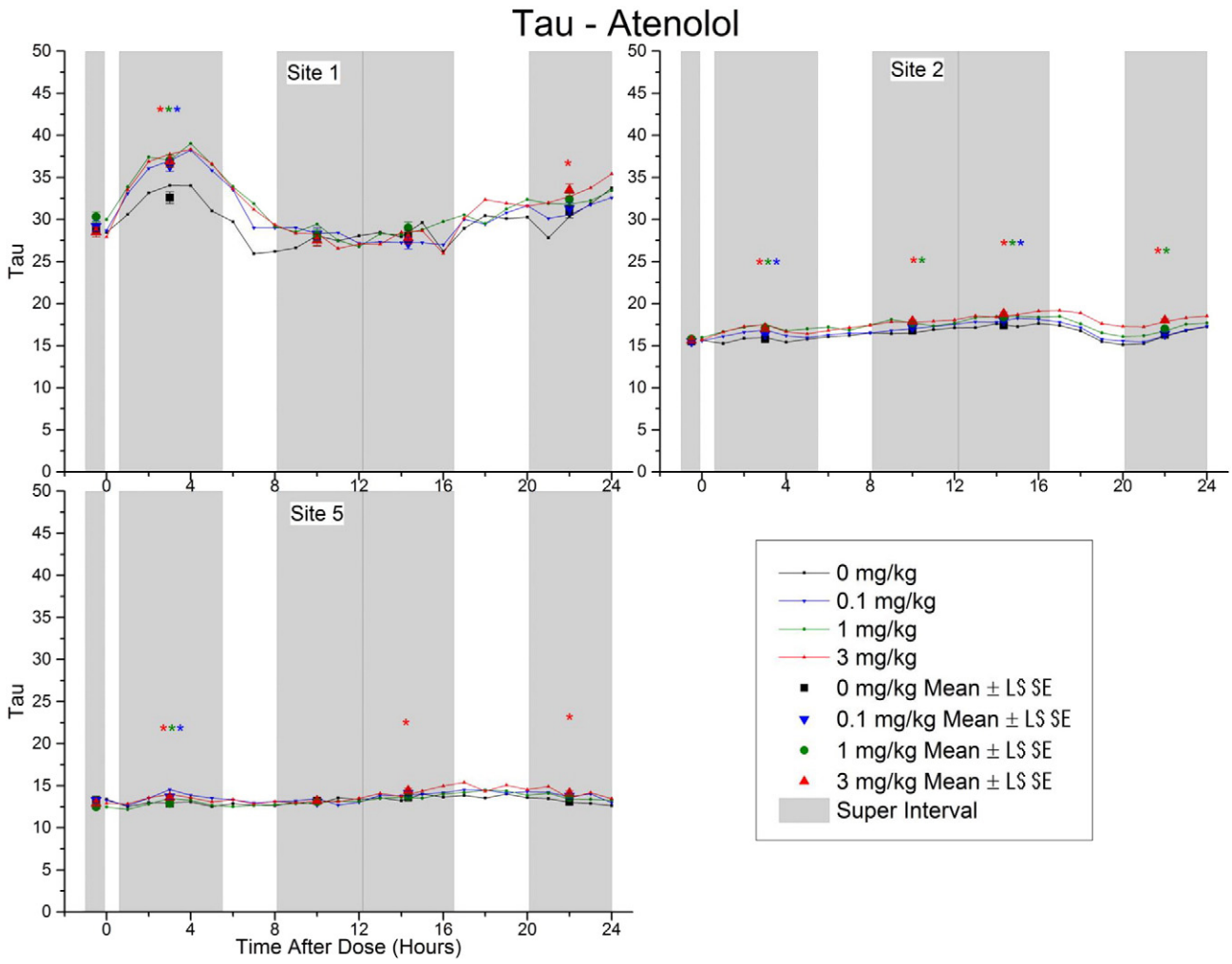

Fig. 17. The effect of atenolol on Tau in conscious instrumented dogs at three different laboratories (sites 1, 2 and 5). Atenolol (vehicle = □, 0.1 mg/kg = ∇, 0.3 mg/kg = ●, 1 mg/kg = △) administered orally at time = 0. Small symbols represent the mean value from the previous 10-min while shaded areas represent super-intervals as defined in Table 3. Large symbols represent the mean ± SE within each super-interval.

5. Conclusion

The data generated by this ILSI/HESI-sponsored consortium has demonstrated that the conscious, chronically instrumented Beagle dog provides a preclinical model capable of detecting drug-induced effects on the inotropic state of the heart. Although $LVdP/dt_{max}$ has been shown recently to provide a robust index of changes in the contractile state of the dog heart (Guth et al., 2015), we have demonstrated that the load-independent measure, $LVdP/dt_{40}$, provided essentially identical results thereby also qualifying it as another index that can be used to assess drug effects on cardiac contractility. In contrast, the QA interval did not react sensitively to the drugs tested. This would not preclude its use in screening methodologies designed to detect large, drug-induced effects; approaches perhaps more appropriate in small animal models. Alternatively, since the QA interval does appear to detect large effects, it could be considered for use in early cardiovascular safety frontloading studies or toxicological studies in which arterial blood pressure and ECG signals are available, again with the caveat that one may only detect large effects on the inotropic state. Although the indices of the lusitropic state of the heart did not respond in a sensitive manner in this study, the drugs tested were not ideally selected to demonstrate effects on these lusitropic parameters in the conscious dog. Additional contractility studies are warranted that provide further insight into integrated physiological effects that examine the lusitropic parameters described in this manuscript using drugs that have robust effects on early diastolic relaxation through calcium handling mechanisms.

Disclaimer

The opinions presented here are those of the authors. No official support or endorsement by the US FDA and participating companies is intended or should be inferred.

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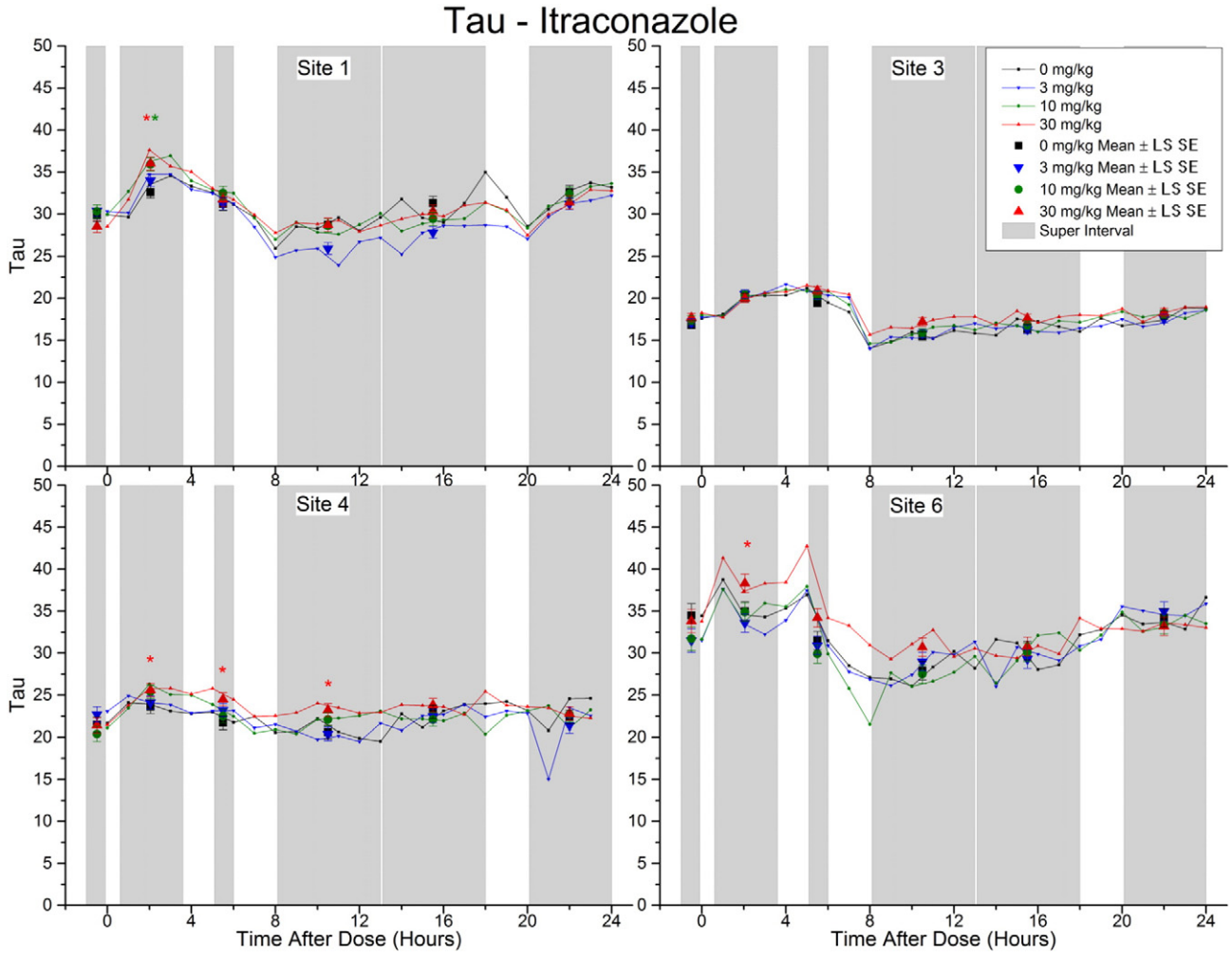

Fig. 18. The effect of itraconazole on Tau in conscious instrumented dogs at four different laboratories (sites 1, 3, 4 and 6). Itraconazole (vehicle = □, 3 mg/kg = ∇, 10 mg/kg = ●, 30 mg/kg = Δ) was administered orally at time = 0. Small symbols represent the mean value from the previous 10-min while shaded areas represent super-intervals as defined in Table 3. Large symbols represent the mean ± SE within each super-interval.

HESI consortium includes representatives of the following companies: AbbVie, Amgen, AstraZeneca, Battelle Memorial Institute, Boehringer Ingelheim, Bristol-Myers Squibb, ChanRx Corporation, Covance, Data Sciences International, Eli Lilly, GE Healthcare, Genentech, GlaxoSmithKline, Hoffman-La Roche, Johnson & Johnson, Lifespan Heart Center, Merck Research Laboratories, Michigan

Table 5
The Least Square (LS) mean blood pressure and heart rate values for each study site at defined super-intervals at the maximal plasma concentration (C_{max}) for both positive and negative inotropic drugs.

	Diastolic blood pressure				Systolic blood pressure				Heart rate			
Amrinone	0	0.5	2	5	0	0.5	2	5	0	0.5	2	5
Site 1	85 ± 1	85 ± 1	80 ± 1	80 ± 1	134 ± 1	133 ± 1	132 ± 1	128 ± 1	68 ± 2	69 ± 2	66 ± 2	68 ± 2
Site 2	81 ± 1	79 ± 1	80 ± 1	79 ± 1	154 ± 1	151 ± 1	154 ± 1	151 ± 1	97 ± 2	96 ± 3	100 ± 2	105 ± 3
Site 3	80 ± 2	76 ± 2	80 ± 2	76 ± 2	130 ± 3	125 ± 2	124 ± 3	121 ± 3	62 ± 3	73 ± 3	80 ± 3	65 ± 3
Site 5	76 ± 3	81 ± 3	85 ± 3	89 ± 3	106 ± 3	107 ± 3	108 ± 3	106 ± 3	91 ± 3	92 ± 4	91 ± 4	113 ± 4
Site 6	92 ± 1	92 ± 1	90 ± 1	88 ± 1	159 ± 1	158 ± 1	159 ± 1	153 ± 2	107 ± 2	108 ± 2	109 ± 2	122 ± 2
Atenolol	0	0.1	1	3	0	0.1	1	3	0	0.1	1	3
Site 1	87 ± 1	85 ± 1	86 ± 1	86 ± 1	139 ± 2	136 ± 2	135 ± 2	138 ± 2	69 ± 3	66 ± 3	65 ± 3	64 ± 3
Site 2	80 ± 1	77 ± 1	77 ± 1	74 ± 1	153 ± 1	150 ± 1	151 ± 1	148 ± 1	92 ± 2	87 ± 2	82 ± 2	79 ± 2
Site 5	83 ± 4	99 ± 4	82 ± 4	78 ± 4	107 ± 4	113 ± 4	110 ± 4	107 ± 4	100 ± 2	88 ± 2	90 ± 2	88 ± 2
Itraconazole	0	3	10	30	0	3	10	30	0	3	10	30
Site 1	87 ± 1	88 ± 1	88 ± 1	92 ± 1	134 ± 2	141 ± 2	134 ± 2	133 ± 2	74 ± 2	68 ± 2	72 ± 2	89 ± 2
Site 3	72 ± 1	78 ± 1	79 ± 1	79 ± 1	134 ± 2	137 ± 2	136 ± 2	126 ± 2	64 ± 2	69 ± 2	73 ± 2	90 ± 2
Site 4	71 ± 1	73 ± 1	73 ± 1	76 ± 1	133 ± 2	134 ± 2	130 ± 2	131 ± 2	84 ± 2	90 ± 2	86 ± 2	100 ± 2
Site 6	91 ± 1	94 ± 1	95 ± 1	97 ± 1	162 ± 2	160 ± 2	162 ± 2	155 ± 2	90 ± 4	103 ± 4	98 ± 4	105 ± 4
Pimobendan	0	0.1	0.3	1	0	0.1	0.3	1	0	0.1	0.3	1
Site 1	80 ± 1	78 ± 1	74 ± 1	70 ± 1	127 ± 2	125 ± 2	122 ± 2	118 ± 2	85 ± 4	84 ± 4	85 ± 4	91 ± 4
Site 2	76 ± 1	76 ± 1	75 ± 1	74 ± 1	150 ± 2	149 ± 2	151 ± 2	147 ± 2	98 ± 3	105 ± 3	99 ± 3	107 ± 2
Site 4	71 ± 1	70 ± 1	70 ± 1	66 ± 1	128 ± 2	129 ± 2	128 ± 2	126 ± 2	109 ± 2	105 ± 2	106 ± 2	110 ± 2

Measures are of the systemic diastolic and systolic arterial BP (mmHg) and left ventricle HR (bpm). Values were derived from the implanted telemetry catheters outlined in Table 2. Blood samples were taken between super-intervals 1 and 2 post-dose.

Table 6
Plasma concentrations determined from multiple laboratories.

Plasma concentration (ng/mL)			
Drug	Low dose	Mid dose	High dose
Amrinone	106 ± 28	453 ± 134	1136 ± 404
Pimobendan	0.302 ± 0.176	0.771 ± 0.270	4.53 ± 4.74
Atenolol	182 ± 98	449 ± 168	1248 ± 149
Itraconazole	251 ± 52	835 ± 81	1993 ± 286

Amrinone plasma concentrations were determined at sites 1, 2, 3, 5 and 6; Pimobendan plasma concentrations were determined at sites 1, 2 and 4; Atenolol plasma concentrations were determined at sites 1, 2 and 5 and Itraconazole plasma concentrations were determined at sites 1, 2, 4 and 6.

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