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Original article

The evaluation of drug-induced changes in cardiac inotropy in dogs: Results from a HESI-sponsored consortium

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ABSTRACT

Introduction: Drug-induced effects on the cardiovascular system remain a major cause of drug attrition. While hemodynamic (blood pressure (BP) and heart rate (HR)) and electrophysiological methods have been used in testing drug safety for years, animal models for assessing myocardial contractility are used less frequently and their translation to humans has not been established. The goal of these studies was to determine whether assessment of contractility and hemodynamics, when measured across different laboratories using the same protocol, could consistently detect drug-induced changes in the inotropic state of the heart using drugs known to have clinically relevant positive and negative effects on myocardial contractility.
Methods: A 4 × 4 double Latin square design (n = 8) design using Beagle dogs was developed. Drugs were administrated orally. Arterial blood pressure, left ventricular pressure (LVP) and the electrocardiogram were assessed. 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, DSI's D70-PCTP system or DSI's Physiotel Digital system. Data acquisition and analysis systems were Ponemah, Notocord or EMKA.
Results: Derived parameters included: diastolic, systolic and mean arterial BP, peak systolic LVP, HR, end-diastolic LVP, and $LVdP/dt_{max}$ as the primary contractility index. Blood samples were drawn to confirm drug exposures predicted from independent pharmacokinetic studies. Across the laboratories, a consistent change in $LVdP/dt_{max}$ was captured despite some differences in the absolute values of some of the hemodynamic parameters prior to treatment.
Discussion: These findings indicate that this experimental model, using the chronically instrumented conscious dog, can accurately and consistently detect changes in cardiac contractility, across multiple sites and instrumentation systems, and that data obtained in this model may also translate to clinical outcomes.

1. Introduction

Drugs can have a variety of effects on the cardiovascular system including hemodynamic and electrophysiological effects. Given the critically important physiological role of the cardiovascular system, in

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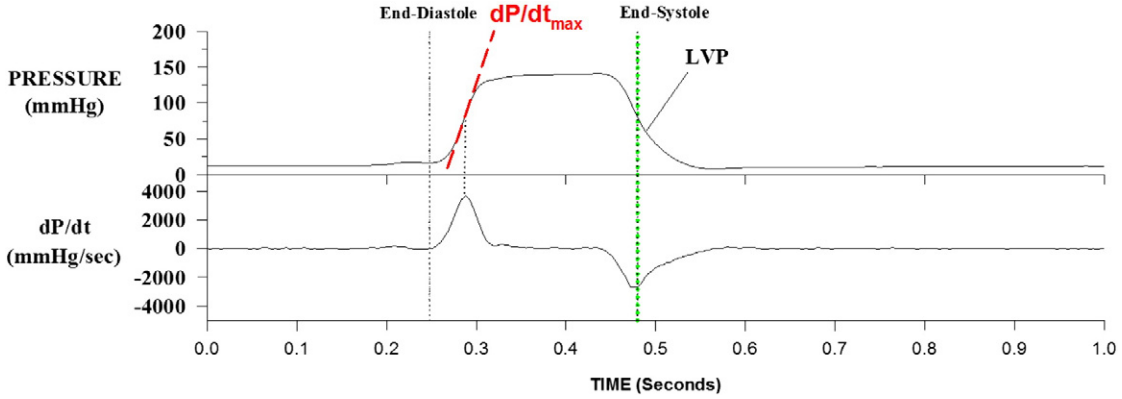

Fig. 1. Left ventricular pressure, its first derivative (dP/dt) and the peak value of dP/dt, (dP/dt_{max}). dP/dt_{max} occurs during systolic contraction when the rate of change in pressure over time (slope of the curve) achieves its peak value. This time can be seen in the top trace where the instantaneous slope of a line tangent to the curve is the highest and in the bottom trace as the peak positive value of dP/dt. (Modified from Sarazan, Kroehle & Main, with permission).

addition to it being an important target for unwanted drug-induced effects, it has long been the subject of nonclinical testing. In 2001, cardiovascular safety assessment was included in a global regulatory guidance for safety pharmacology testing (Anon., 2001). A fundamental property of the cardiovascular system is the inotropic (or contractile) state of the heart (these two terms will be used interchangeably in this manuscript), which is a major determinant of cardiac output. The inotropic state of the heart is under neurohumoral control to match the cardiac output to the metabolic needs of the body. However, the inotropic state of the heart may be affected by disease and is also a potential target for drug-induced effects, with direct influence on cardiac pump performance and therefore cardiac output.

Both increases and decreases in the inotropic state of the heart may be harmful, depending upon the patient population. Whereas a slight change of the inotropic state of the heart may not compromise cardiac output in a healthy individual, a patient with depressed cardiac pump performance may be subject to an acute heart failure and dyspnea. Alternatively, an increase in the inotropic state of the heart may not be tolerated by a patient with coronary artery disease since the resultant increase in myocardial oxygen consumption, and therefore demand for myocardial perfusion, may not be met, resulting in a mismatch in oxygen demand and supply. Thus, it should be obvious that unwanted, drug-induced alterations of the inotropic state of the heart can pose a risk to patients.

Given the potential for drug-induced effects on the myocardial inotropic state to be clinically relevant in susceptible patient populations, it is advisable to evaluate drugs for such effects when targeted for susceptible patients. While it seems surprising that examinations of the inotropic effects of drugs are not a part of the core battery studies outlined in the ICH S7A Guidance on Safety Pharmacology, in large part this was due to the idea that first in human studies would primarily be conducted in healthy subjects, and the core battery was put in place to prepare for those studies. Additionally, at the time the guideline was written, there was the perception that there was a lack of a practical experimental approach to test for such effects. Indeed, the assessment of the inotropic state of the heart is fraught with challenges. Theoretically, the preferred approach for making this assessment is based on the wall stress versus strain relationship of the heart throughout the cardiac cycle, which can be estimated using the relationship between the ventricular pressure versus volume (Suga, Sagawa, & Shoukas, 1973). The utility of this approach for routine drug testing is limited, however, since the continuous measurement of ventricular volume over time, particularly in conscious animals, is challenging. An alternative approach for assessing the inotropic state of the heart uses the maximal rate of pressure increase in the left ventricle during systole ($LVdP/dt_{max}$). This parameter is experimentally accessible since it only requires a high fidelity left ventricular pressure signal, which can be obtained through the use of a high fidelity left ventricular pressure transducer, and a high frequency differentiator (Fig. 1). Although this index of myocardial contractility can be affected by changes in both left ventricular preload and afterload, in addition to changes in inotropic state (Hamlin & del Rio, 2012), studies have consistently demonstrated

Table 1
Drugs tested in conscious telemetered dogs for effects on left ventricular contractility.

Test article	Doses (oral)	Formulation/vehicle	Cardiac inotropic effect
Pimobendan	Vehicle, 0.1, 0.3 & 1 mg/kg	PCCA Fixed Oil Suspension Vehicle™	Positive
Amrinone	Vehicle, 0.5, 2 & 5 mg/kg	Dosed in gelatin capsules	Positive
Itraconazole	Vehicle, 3, 10 & 30 mg/kg	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)	Negative
Atenolol	Vehicle, 0.3, 1 & 3 mg/kg	Deionized water	Negative

Table 2
Study site characteristics and drugs tested in contractility assessments by participating companies (listed alphabetically).

Participant	No. of Beagle dogs	Strain	Source	Sex	Telemetry system	Software
AbbVie	8	Beagle	Marshall Farms	Male	DSI Physiotel D70-PCTP	Emka
Amgen/Covance	8	Beagle	Covance Research Products	Male	DSI Physiotel D70-PCTP	Ponemah
Boehringer Ingelheim	8	Beagle	Marshall/Boehringer Ingelheim	M/F	Konigsberg (ITS) T27	Notocord
Data Sciences International (DSI)	8	Beagle	Covance Research Products	Male	DSI Physiotel Digital L21	Ponemah
Millennium	8	Beagle	Marshall BioResources	Male	DSI Physiotel D70-PCTP	EMKA
Sanofi	8	Beagle	Harlan	M/F	DSI Physiotel D70-PCTP	Notocord

Table 3
Super-intervals evaluated for each of the test compounds (in hours after dosing).

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

that ventricular preload affects LVdP/dt$_{max}$ only in extreme situations (Zimpfer & Vatner, 1981) and LVdP/dt$_{max}$ is remarkably free from effects of afterload (Maso, Braunwald, Covell, Sonnenblick, & Ross, 1971). Furthermore, acute, drug induced changes in preload or afterload would be easily detected through changes in left ventricular end diastolic pressure or systemic arterial pressure, respectively. LV dP/dt$_{max}$ is, however, highly dependent on heart rate, such that drug-induced changes in heart rate will also affect LVdP/dt$_{max}$. Keeping in mind LVdP/dt$_{max}$'s potential use in drug testing, one would not anticipate such large changes in either preload, afterload or heart rate that could complicate the interpretation of drug-induced effects on this index since large changes in these parameters would per se pose serious concerns of a given compound's suitability for development. Thus, LVdP/dt$_{max}$ may offer a useful parameter for detecting drug-induced inotropic activity in the conscious animal.

The use of LVdP/dt$_{max}$ as an index of cardiac contractility in drug testing would be further supported if it could be shown to provide similar data describing drug-induced effects across multiple laboratories (Sarazan et al., 2011). The relationship between plasma drug concentration and its inotropic effect in the animal should also be similar to that seen in humans, if the model is to be useful in predicting risk to patients. The ILSI Health and Environmental Sciences Institute (HESI) has therefore organized a multi-center consortium with this goal in mind. The participants have designed a double 4 × 4 Latin square study (n = 8/site) using chronically instrumented Beagle dogs to assess dose-dependent, drug-induced LV contractility changes after treatment with drugs known to have clinically relevant positive (pimobendan and amrinone) or negative (itraconazole and atenolol) inotropic effects (Table 1). Additionally, arterial blood pressure (BP), LV pressure (LVP) and ECG were collected using telemetry prior to dosing and over

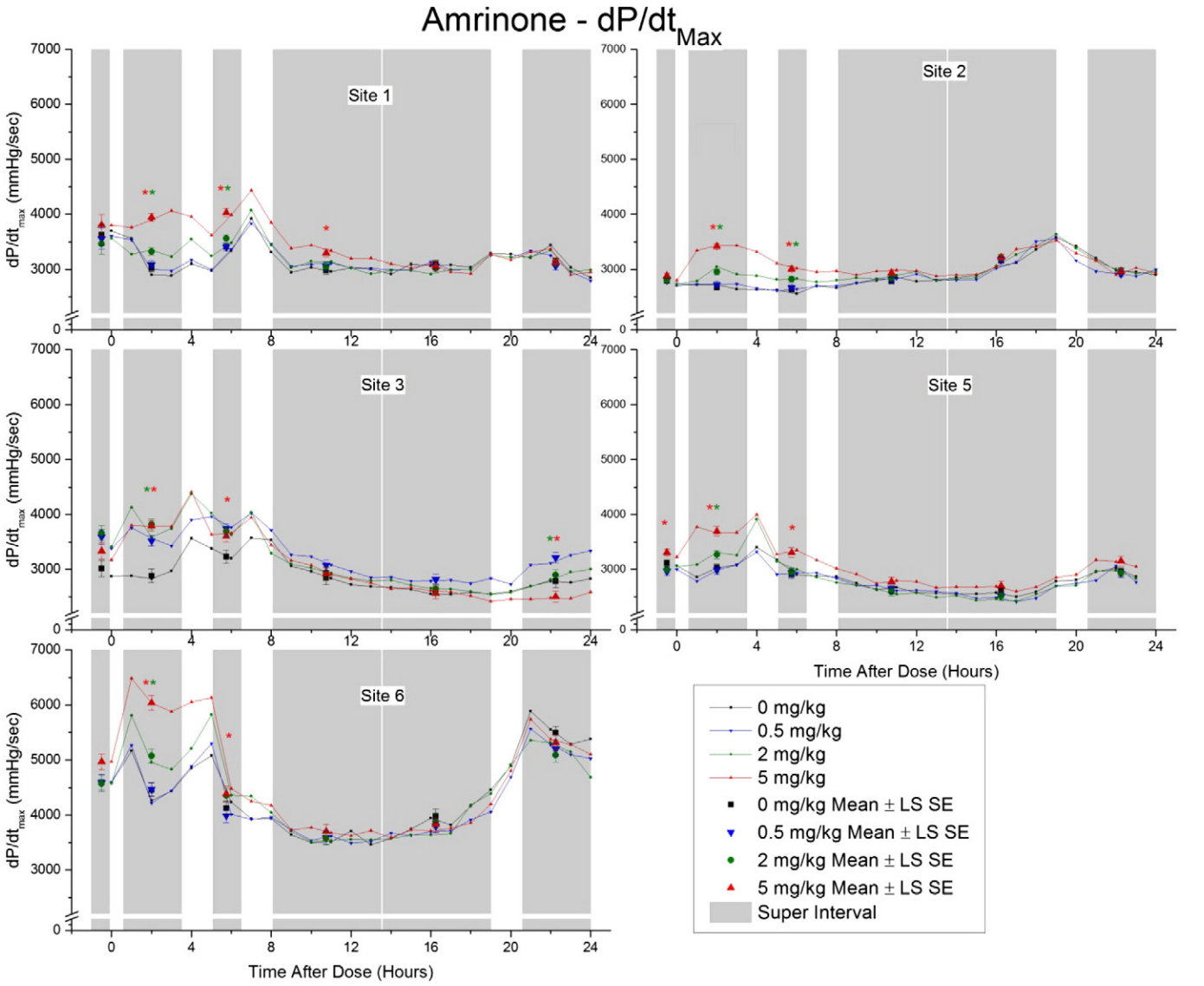

Fig. 2. Effect of amrinone on LV dP/dt$_{max}$ in conscious instrumented dogs at five different laboratories. Amrinone (vehicle = □, 0.5 mg/kg = ∇, 2 mg/kg = •, 5 mg/kg = Δ) administered orally at time = 0. Small symbols represent the mean value from the previous 10-minutes. Shaded areas represent super-intervals as defined in Table 3. Large symbols represent the mean ± SE within each super-interval.

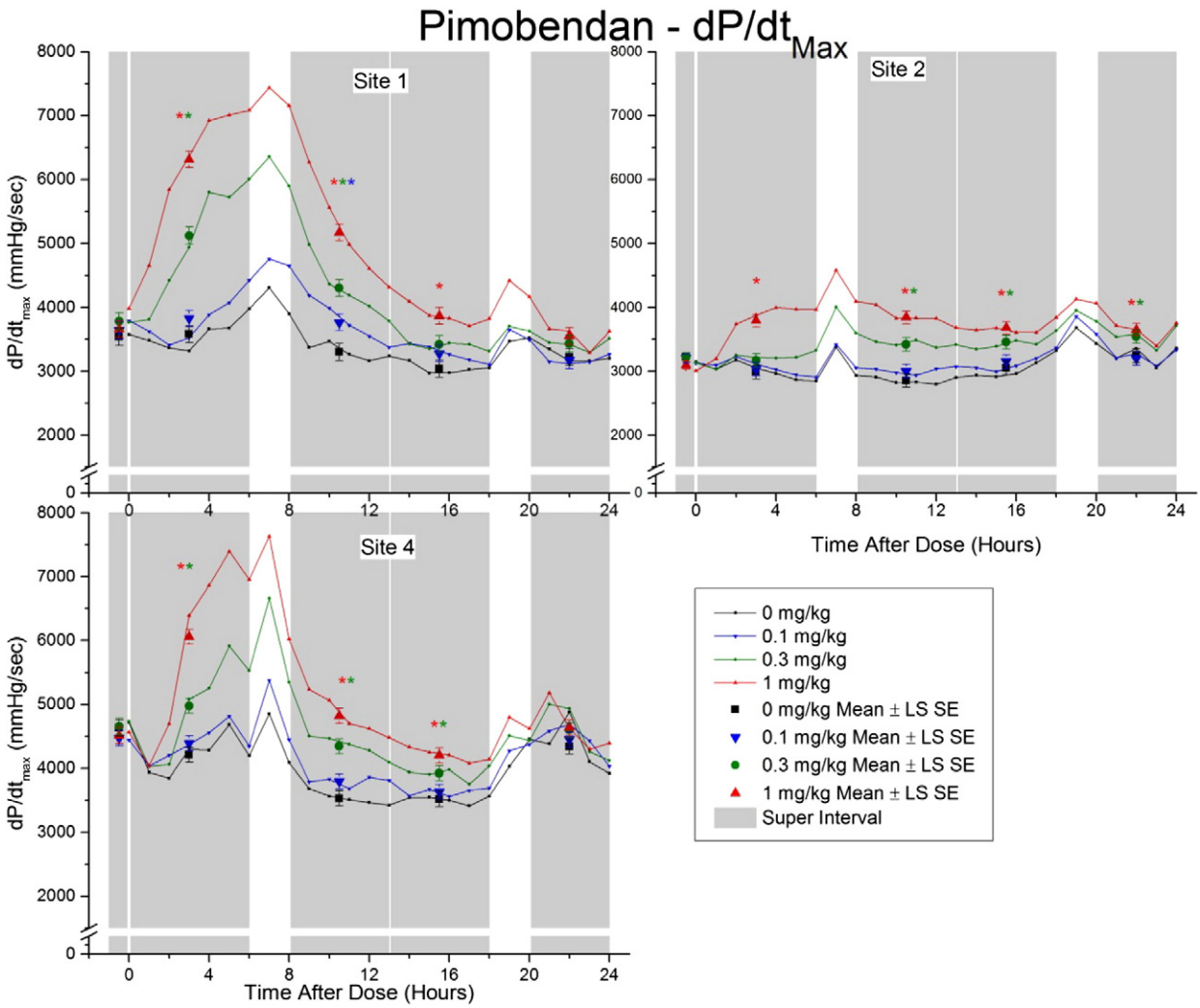

Fig. 3. Effect of pimobendan on LV dP/dt_{max} in conscious instrumented dogs at three different laboratories. Pimobendan (vehicle = □, 0.1 mg/kg = ∇, 0.3 mg/kg = •, 1 mg/kg = Δ) administered orally at time = 0. Small symbols represent the mean value from the previous 10-minutes. Shaded areas represent super-intervals as defined in Table 3. Large symbols represent the mean ± SE within each super-interval.

a 24 h period post-dosing. Derived parameters in addition to $LVdP/dt_{max}$, included peak systolic LVP, HR, systolic, diastolic and mean arterial blood pressure, the Q-A interval (Norton, Iacono, & Vezina, 2009), left ventricular end-diastolic pressure (LVEDP), dP/dt_{min}, and Tau (Weiss, Frederiksen, & Weisfeldt, 1976). In this report, only the $LVdP/dt_{max}$ parameter will be included, whereas the other measures of systolic function (Q-A interval and dP/dt_{40}) and lusitropic endpoints (dP/dt_{min}, and Tau) will be dealt with in a subsequent report.

The data derived indicate that $LVdP/dt_{max}$ is a robust, sensitive and reliable parameter, useful for assessing both clinically relevant drug-induced positive and negative inotropic effects. Despite differences in baseline values, there was good reproducibility of results across the participating laboratories suggesting that it can be successfully applied across laboratories despite these differences. Importantly, plasma drug levels achieved in these studies were similar to those having clinically relevant effects. This suggests a good translation of the effects from the conscious dog model to effects seen in man, thereby supporting the value of these studies in drug development.

2. Materials and methods

2.1. Test facilities

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 outsourced to a contract research 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 given priority when conflicts arose.

2.2. Experimental animals

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

Animals were either naïve at the study onset (one site) or they had been used previously in safety pharmacology studies but were healthy and free of any residual test article at the start of the study (remaining 5 sites). 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,

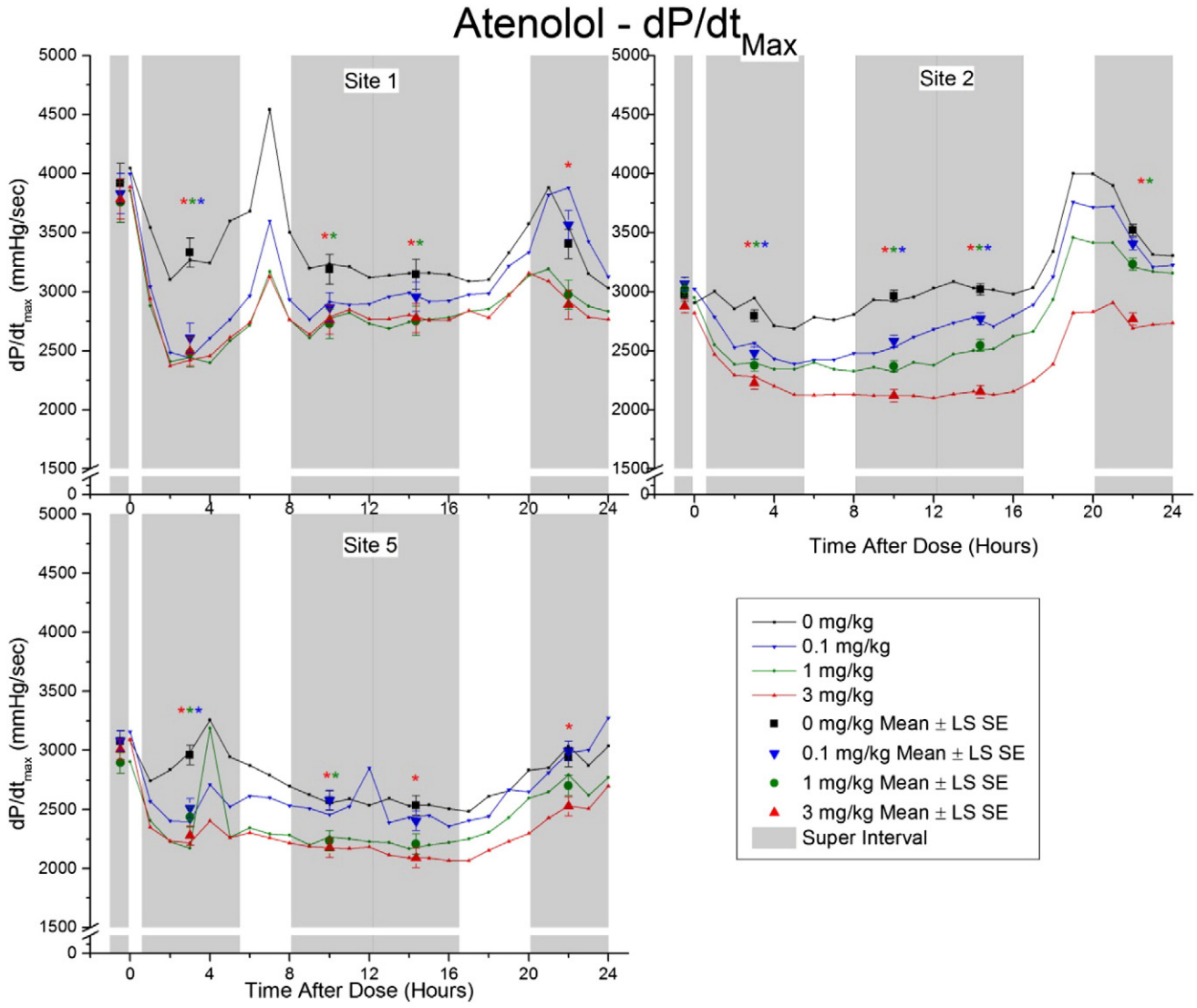

Fig. 4. Effect of atenolol on LV dP/dt_{max} in conscious instrumented dogs at three different laboratories. Atenolol (vehicle = □, 0.1 mg/kg = ∇, 0.3 mg/kg = •, 1 mg/kg = Δ) administered orally at time = 0. Small symbols represent the mean value from the previous 10-minutes. Shaded areas represent super-intervals as defined in Table 3. Large symbols represent the mean ± SE within each super-interval.

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 blood pressure (BP), left ventricular pressure (LVP), the electrocardiogram (ECG), body temperature and activity (note 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's 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 the institutions' approved procedures. All animals were allowed to fully recover from surgery prior to study onset. In some cases, dogs were obtained from a preexisting colony of instrumented animals that had previously been used for other 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.

The anesthetized dogs were placed in right lateral recumbency. 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 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

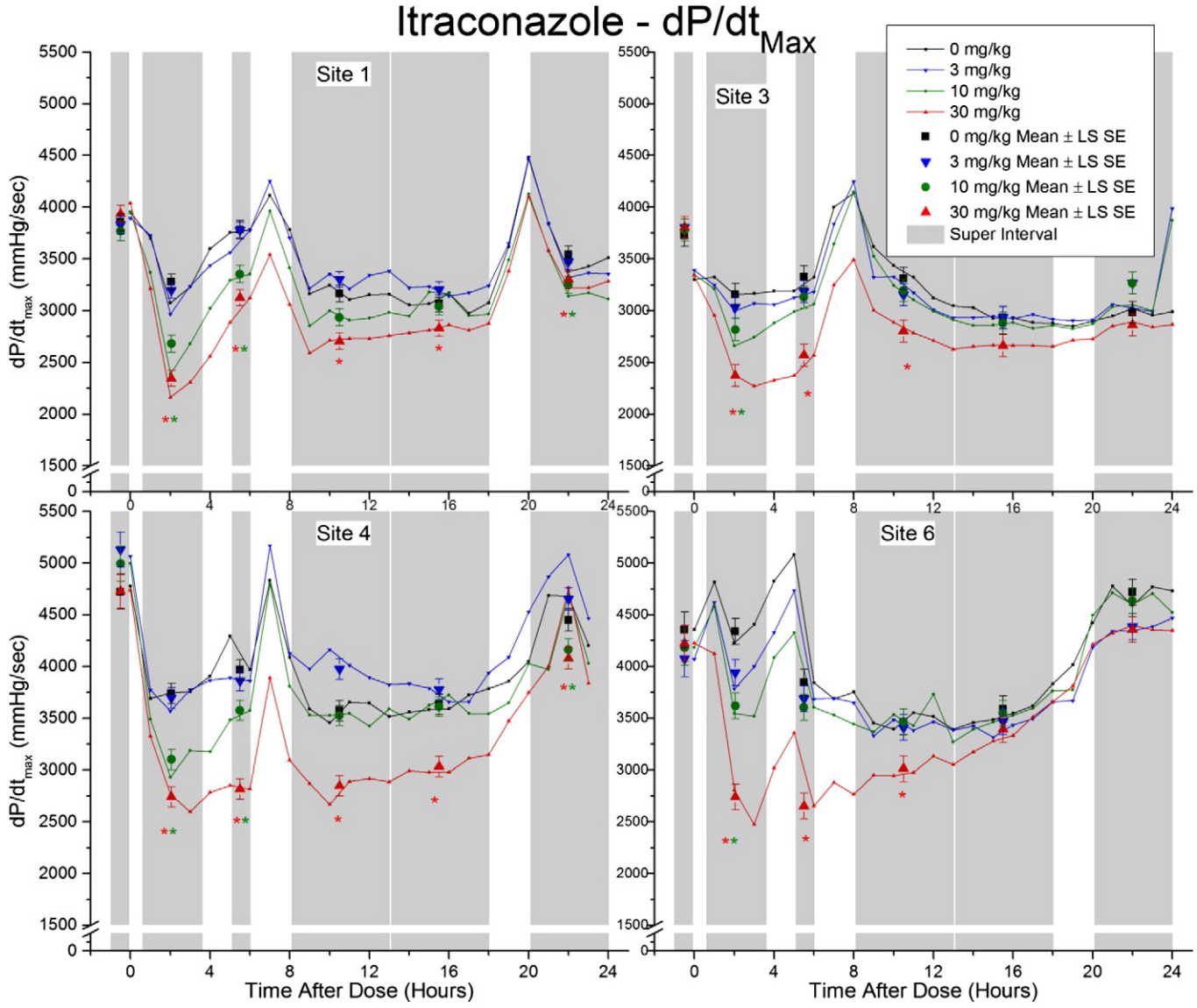

Fig. 5. Effect of itraconazole on LV dP/dt_{max} in conscious instrumented dogs at four different laboratories. Itraconazole (vehicle = □, 3 mg/kg = ∇, 10 mg/kg = •, 30 mg/kg = Δ) administered orally at time = 0. Small symbols represent the mean value from the previous 10-minutes. Shaded areas represent super-intervals as defined in Table 3. Large symbols represent the mean ± SE within each super-interval.

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 transducer, which was then secured with a purse string suture.

A purse string suture was preplaced in the descending thoracic aorta, the aorta was 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 appropriate recovery period defined by each participating laboratory, animals were returned to the colony of implanted dogs in group-housing conditions.

Table 4
Summary of the pharmacokinetics of the four drugs tested as determined in independent studies.

Compound	Dose (mg/kg)	T_{max} (hours)	C_{max} (ng/mL)	AUC (ng * hr/mL)
Amrinone	0.5	1.7 ± 0.5	269 ± 64	1247 ± 152
	2	2.7 ± 1.0	743 ± 257	4448 ± 1265
	5	2.5 ± 1.2	2652 ± 1096	13,795 ± 4264
Atenolol	0.3	1.3 ± 0.5	174 ± 53	997 ± 143
	1	1.5 ± 0.5	538 ± 92	3333 ± 482
	3	1.3 ± 0.5	1718 ± 364	9425 ± 1026
Itraconazole	3	2.5 ± 1.2	373 ± 92	6748 ± 1605
	10	2.3 ± 0.8	1253 ± 222	23,083 ± 5184
	30	2.7 ± 2.2	2127 ± 838	47,450 ± 13,794
Pimobendan	0.1	3.3 ± 1.0	1.9 ± 0.6	4.4 ± 1.6
	0.3	3.2 ± 1.3	7.3 ± 2.7	22.5 ± 10.4
	1	2.3 ± 1.4	22.9 ± 13.5	89.7 ± 17.8

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.

Table 5
Average plasma drug concentrations (ng/mL) measured in each study at a single time point to confirm drug exposure. For oral doses used refer to Table 1.

Amrinone		Low dose	Mid dose	High dose
	Site 1	74	304	588
	Site 2	NA	NA	NA
	Site 3	122	485	1390
	Site 5	135	621	1484
	Site 6	92	402	1080
Average		106	453	1136
Pimobendan				
	Site 1	0.505	1.08	10.0
	Site 2	0.205	0.582	1.771
	Site 4	0.195	0.65	1.825
Average		0.317	0.77	4.53
Atenolol				
	Site 1	251	639	1409
	Site 2	NA	320	1114
	Site 5	112	389	1221
Average		182	449	1248
Itraconazole				
	Site 1	199	877	1760
	Site 3	316	908	2230
	Site 4	267	722	1730
	Site 6	220	832	2250
Average		251	835	1993

The abdominal surgical implantation procedure for DSI implants was similar across all sites and device models. Differences included: 1) placement of the implant body either within the peritoneal cavity or in a pocket between the peritoneum and the abdominal muscles, 2) the L21 implant has a short external antenna that was routed between the peritoneum and the abdominal muscles, whereas the D70-PCTP's antenna is internal, and 3) some blood pressure sensor cannulae were inserted into a femoral artery and others were inserted into a branch of the mesenteric artery that perfuses the jejunum. In both cases, the artery or arterial branch was ligated and the sensor tip was advanced into the abdominal aorta. The implants arrived precalibrated 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 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 hole 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 on the computer monitor, the purse string suture was tightened and tied using traditional surgical knot tying technique. 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. Not all test articles were studied in each laboratory (Table 2). The doses and formulations used are described in Table 1.

2.6. Study design

Four different treatments were administered to each dog in the order prescribed by a double 4 × 4 Latin square design (n = 8) over four treatment days, with an appropriate washout period between days. A 4 × 4 Latin square contains four rows and four columns. The four treatments are randomly assigned to experimental units within the rows (days) and columns (animals) so that each treatment appears in every row and in every column. A double 4 × 4 Latin square is a combination of two identical 4 × 4 Latin squares, resulting 4 rows and 8 columns. The design is particularly appropriate for comparing four treatment means in the presence of two sources of extraneous variation. The washout period was a minimum of 72 h between treatment days.

Food was withdrawn approximately 2 h before dosing in the morning and reintroduced in the afternoon, which was well after the anticipated t_{max} of the tested drug.

2.7. Pharmacokinetic profiles and exposure confirmation

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

2.8. Data collection and analysis

2.8.1. Raw data (signals)

Digital cardiac left ventricular pressure (LVP), aortic blood pressure (BP), and electrocardiogram (ECG) signals were continuously acquired from at least 1 h prior to dosing through 24 h post-dose on each study day. In some cases body temperature and activity signals were also acquired. Sampling rates were ≥500 Hz for LVP and ECG signals, ≥250 Hz for BP signals and ≥20 Hz for temperature and activity signals, which is adequate for the frequency content of each of these signal types (Sarazan, 2014; Sarazan, Kroehle, & Main, 2012). 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 at each study site and included: heart rate, diastolic aortic pressure, systolic aortic pressure, mean aortic pressure, pulse pressure, left ventricular end-diastolic pressure, peak systolic left ventricular pressure, dP/dt_{max}, dP/dt_{min}, dP/dt_{40}, Tau (or the left ventricular diastolic time constant) and the Q-A interval (or systolic time interval between the Q wave and the onset of the aortic blood pressure 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 cardiac contractility.

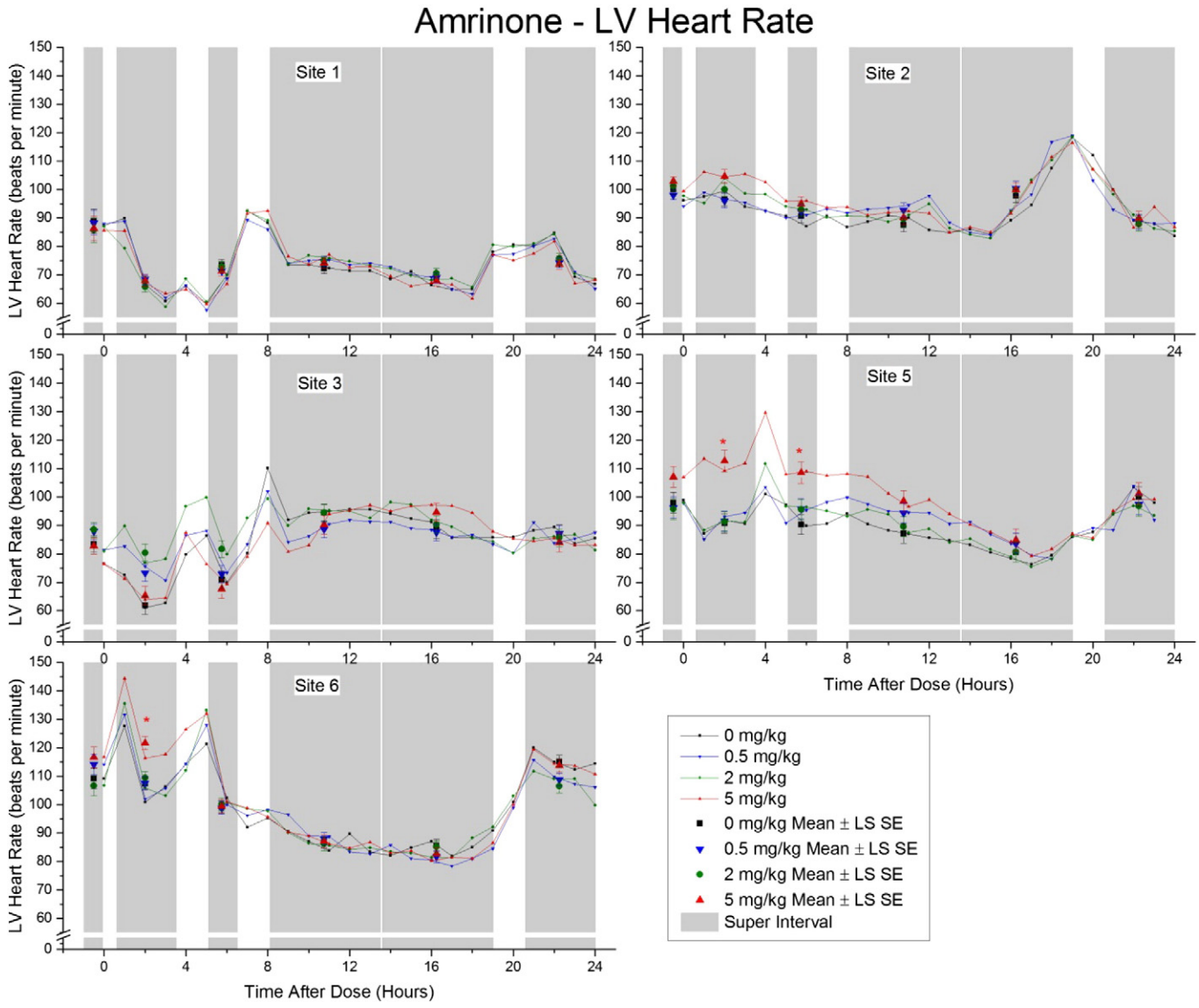

Fig. 6. Effect of amrinone on heart rate in conscious instrumented dogs at five different laboratories. Amrinone (vehicle = □, 0.5 mg/kg = ∇, 2 mg/kg = •, 5 mg/kg = Δ) administered orally at time = 0. Small symbols represent the mean value from the previous 10-minutes. Shaded areas represent super-intervals as defined in Table 3. Large symbols represent the mean ± SE within each super-interval.

2.8.3. Statistical analysis

Although derived data were calculated for every cardiac cycle, the results were collapsed into 10-minute mean values for further analysis. These mean values were further averaged to construct the pre-specified super-intervals (Sivarajah et al., 2010) for each test article as shown in Table 3. The super-intervals used for each compound were defined by a data evaluation subteam prior to the statistical analysis. The selection was intended to avoid disturbances associated with dosing, changes in light cycle or at the time of blood sampling for exposure confirmation. Each compound was treated individually, selecting intervals from the average of $LVdP/dt_{max}$ across the laboratories that tested a given compound.

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, (pretreatment) 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. Nonmonotonic dose responses 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 primary goal of this study was to assess whether multiple laboratories, using similar but not identical state-of-the-art approaches for measuring cardiovascular function in the conscious dog, were capable of producing similar experimental results on the inotropic effects of the compounds tested. The parameter $LVdP/dt_{max}$ was selected to be the most promising for this purpose and those results are presented in this first manuscript. A second, but important factor to interpret results relates to the relationship of the observed effect on myocardial contractility to the plasma drug levels of the compounds tested. This is

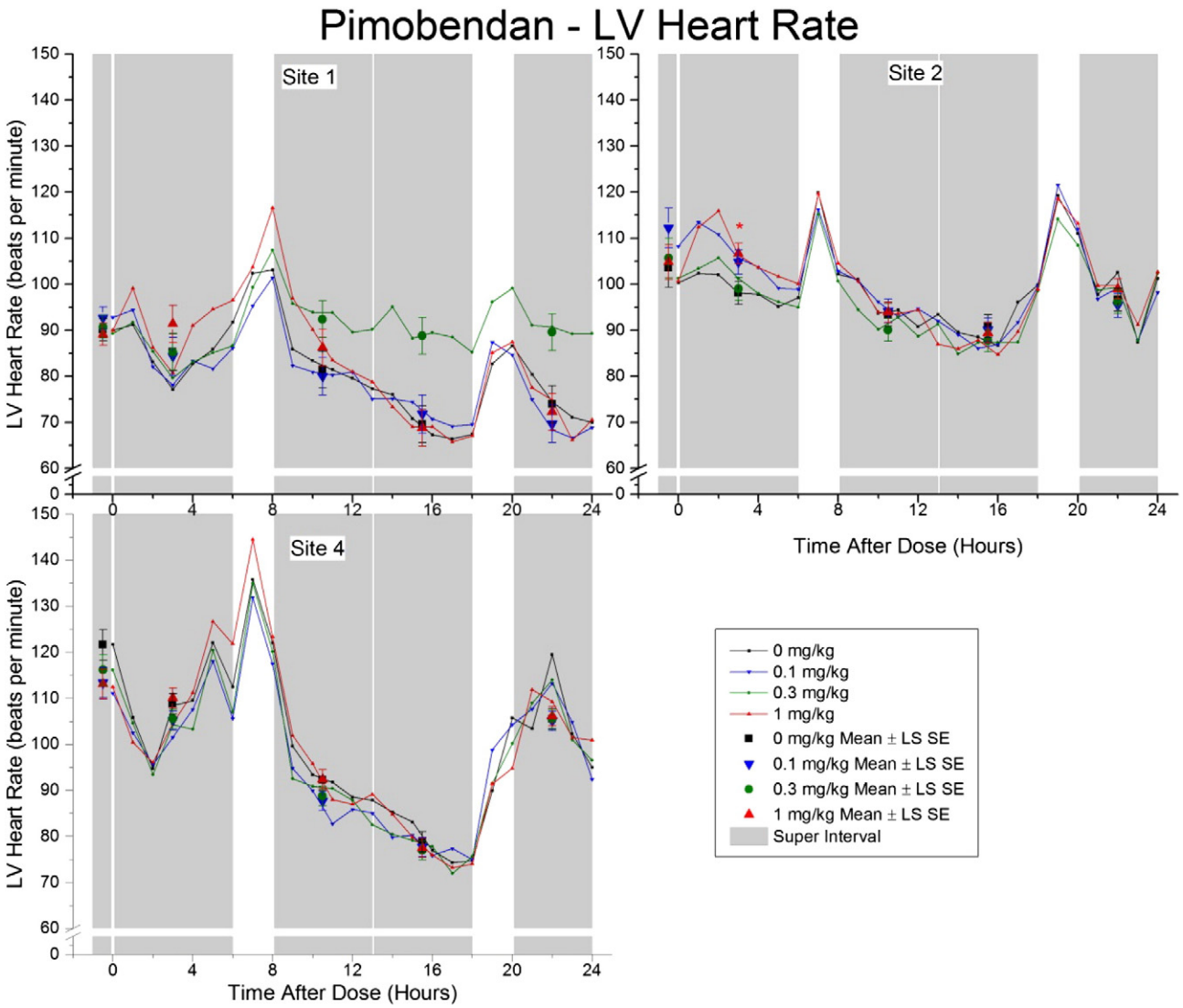

Fig. 7. Effect of pimobendan on heart rate in conscious instrumented dogs at three different laboratories. Pimobendan (vehicle = □, 0.1 mg/kg = ▽, 0.3 mg/kg = •, 1 mg/kg = △) administered orally at time = 0. Small symbols represent the mean value from the previous 10-minutes. Shaded areas represent super-intervals as defined in Table 3. Large symbols represent the mean ± SE within each super-interval.

important to establish the degree of translation between PK (plasma drug level) and PD (effect on inotropic state) in the dog model used in this study, in comparison to the PK/PD relationship found with clinical use of the drugs, as reported in the literature. As mentioned above, the parameter LVdP/dt$_{max}$ can be influenced by changes in heart rate or either preload or afterload of the left ventricle. The parameters measured in these studies that reflect these potential changes, due to their effect on LVdP/dt$_{max}$, were also examined.

3.1. Effects of the test compounds on LVdP/dt$_{max}$ across laboratories

Studies using amrinone were conducted in 5 different laboratories, atenolol and pimobendan were each tested in 3 laboratories and itraconazole was tested in 4 different laboratories. The dose- and time-dependent effects of the four test compounds on LVdP/dt$_{max}$, as detected in each laboratory, are summarized in Figs. 2–5, respectively.

3.2. Positive inotropic drugs

3.2.1. Amrinone

All five laboratories that tested amrinone (Fig. 2) detected a dose-dependent and reversible increase in LVdP/dt$_{max}$ following both the mid (2 mg/kg) and high (5 mg/kg) doses. None of the laboratories found an effect on LVdP/dt$_{max}$ with the low dose of 0.5 mg/kg. A slight difference was seen between labs with regards to the duration of the effect, with effects being seen in all labs during both the first and second super-intervals post-dosing with the high dose, but with increases following the mid dose not lasting into the second superinterval in all labs. One of the labs observed significant increases in LVdP/dt$_{max}$ following the high dose even into the 3rd superinterval (8–13.5 h post-dosing).

It was noted that the absolute values of LVdP/dt$_{max}$ prior to administration were variable amongst the laboratories, ranging from 2885 to 5142 mm Hg/s (Fig. 2). This different baseline level appeared to have no effect on the sensitivity of the model to detect the drug-induced increase, however.

3.2.2. Pimobendan

The three laboratories that tested pimobendan found a dose-dependent and reversible increase in LVdP/dt$_{max}$ following both the mid (0.3 mg/kg) and the high doses (1.0 mg/kg) (Fig. 3). With the low dose there was no significant effect on LVdP/dt$_{max}$ in any of the three studies. The increase in LVdP/dt$_{max}$ was long-lasting, being significant

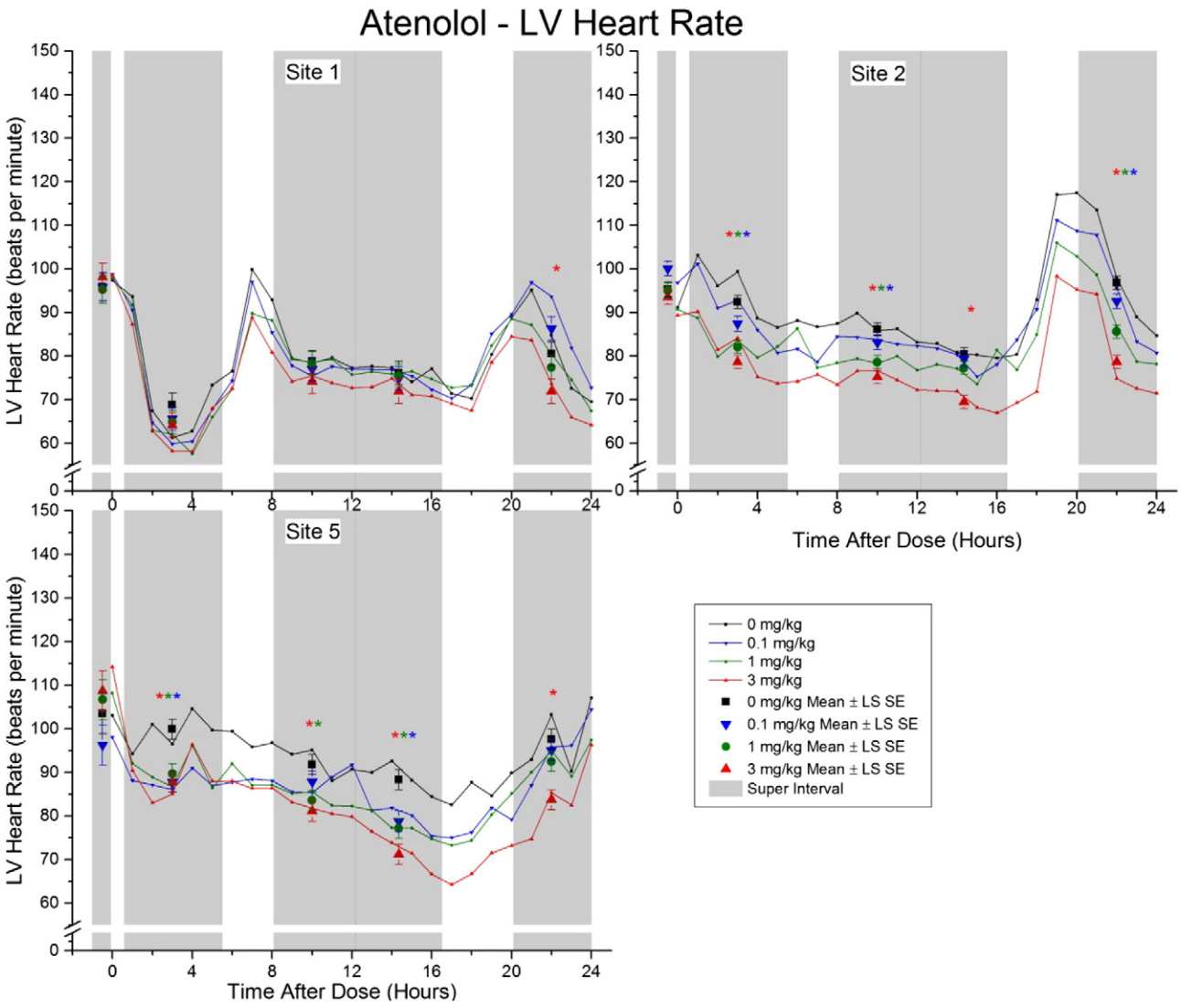

Fig. 8. Effect of atenolol on heart rate in conscious instrumented dogs at three different laboratories. Atenolol (vehicle = □, 0.1 mg/kg = ∇, 0.3 mg/kg = •, 1 mg/kg = Δ) administered orally at time = 0. Small symbols represent the mean value from the previous 10-minutes. Shaded areas represent super-intervals as defined in Table 3. Large symbols represent the mean ± SE within each super-interval.

during the last superinterval (20–24 h post-dosing) in one of the three studies (site 2).

It was once again noted that differences in baseline values for $LVdP/dt_{max}$ had no apparent impact on the ability of the model to detect the drug-induced effects. Also, the absolute magnitude of the increase in $LVdP/dt_{max}$ was similar in two of the laboratories (sites 1 and 4), but less in the third (site 2).

3.3. Negative inotropic drugs

3.3.1. Atenolol

Three laboratories studied the effect of atenolol with two of them finding a significant decrease in $LVdP/dt_{max}$ with all three doses tested (0.3, 1, and 3 mg/kg) and for all of the 4 super-intervals defined (Fig. 4). The third laboratory did not detect a significant decrease in $LVdP/dt_{max}$ with the lowest dose, during any of the super-intervals, despite the fact that the mean values of $LVdP/dt_{max}$ following treatment were consistently below the baseline values particularly in the first super interval (0.5–5.5 h post-dosing).

3.3.2. Itraconazole

Itraconazole was examined in four different laboratories and the results are summarized in Fig. 5. All four laboratories detected a dose-dependent and reversible decrease in $LVdP/dt_{max}$, most markedly with the high dose of 30 mg/kg, but also with the mid dose of 10 mg/kg in some cases. None of the labs detected an effect on $LVdP/dt_{max}$ with the low dose of 3 mg/kg. The effect was long-lasting but returned largely to control values by 24 h post-dosing, although slight effects still persisted at two labs (site 1 and site 4).

3.4. Plasma drug levels

The four test compounds were tested in dedicated pharmacokinetic studies prior to the main studies 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 drawing blood for exposure confirmation in the main studies, as well as for defining the super-intervals for the statistical evaluation of the data. The summary data from these 4 pharmacokinetic studies are summarized in Table 4.

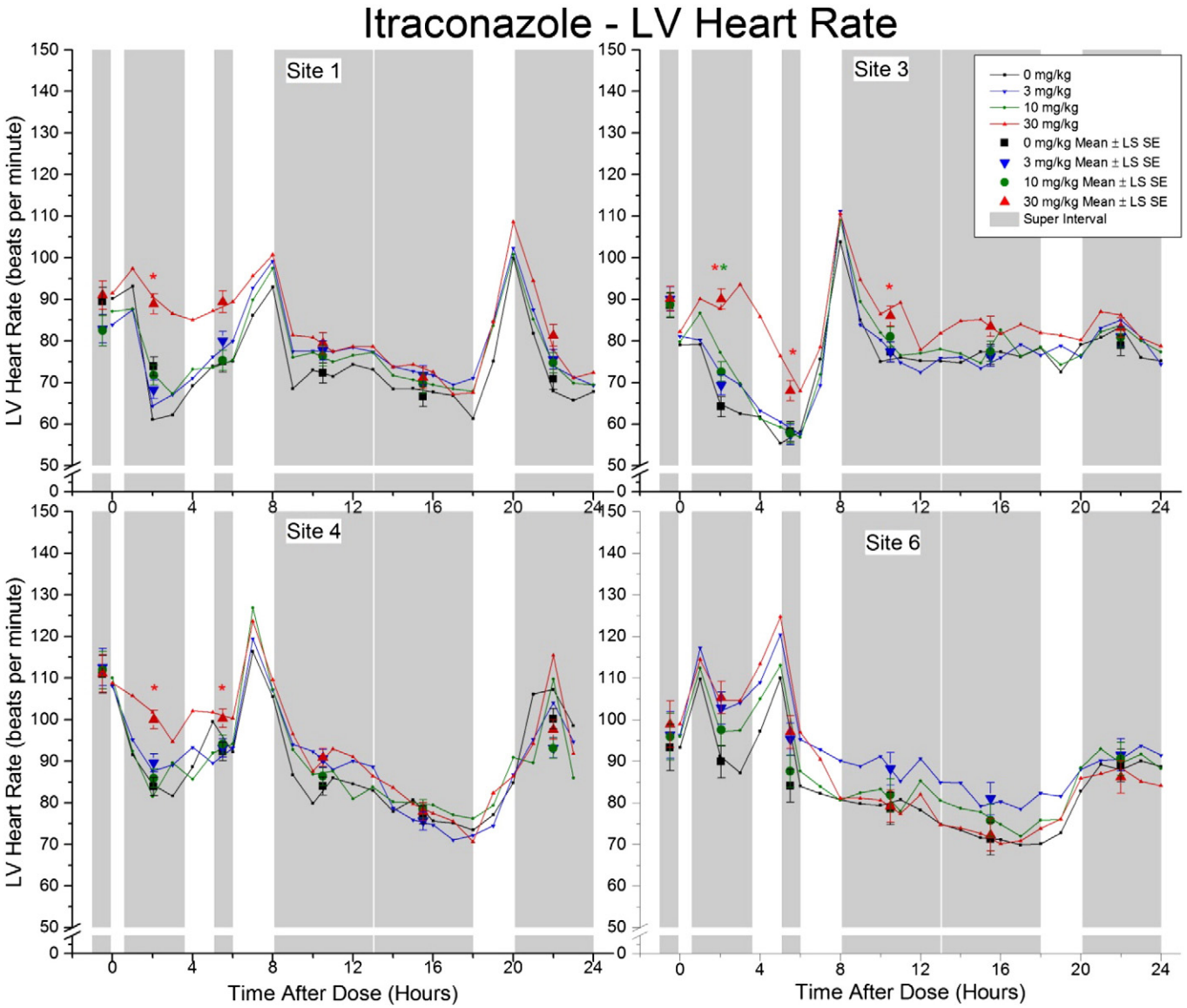

Fig. 9. Effect of itraconazole on heart rate in conscious instrumented dogs at four different laboratories. Itraconazole (vehicle = □, 3 mg/kg = ∇, 10 mg/kg = •, 30 mg/kg = Δ) administered orally at time = 0. Small symbols represent the mean value from the previous 10-minutes. Shaded areas represent super-intervals as defined in Table 3. Large symbols represent the mean ± SE within each super-interval.

3.5. Positive inotropic drugs

3.5.1. Amrinone

When given in oral doses of 0.5, 2 or 5 mg/kg, amrinone lead to peak plasma levels (C_{max}) of 269, 743 and 2652 ng/mL, respectively (Table 4). The time of the maximal plasma concentration (T_{max}) was similar ranging from 1.7 to 2.7 h post-dosing.

3.5.2. Pimobendan

Pimobendan was tested using oral doses of 0.1, 0.3 and 1.0 mg/kg. The resulting C_{max} levels were 1.9, 7.3 and 22.9 ng/mL occurring at T_{max} of 3.3, 3.2 and 2.3 h post-dosing, respectively (Table 4).

3.6. Negative inotropic drugs

3.6.1. Atenolol

Oral doses of atenolol were 0.3, 1 and 3 mg/kg and led to C_{max} values of 174, 538 and 1718 ng/mL occurring at T_{max} values of between 1.3 and 1.5 h post-dosing (Table 4).

3.6.2. Itraconazole

Doses for itraconazole were 3, 10 and 30 mg/kg given orally and these were associated with C_{max} values of 373, 1253 and 2127 ng/mL, respectively (Table 4). The T_{max} values were between 2.3 and 2.7 h post-doing.

In most studies, plasma samples were drawn at a single time point to confirm that animals in the study had drug exposures in the anticipated range based on the preliminary pharmacokinetic studies. These data are summarized in Table 5 and confirm the expected exposure, as well as a good consistency of exposures across laboratories. It should be kept in mind, however, that the exposure data do not represent maximal drug concentrations as determined in the pharmacokinetic studies, as the timing of the blood draw was specified to be after T_{max} to avoid artifacts in the hemodynamic parameters at the time of maximal plasma levels.

3.7. Hemodynamic parameters affecting $LVdP/dt_{max}$

The primary parameter used to assess myocardial contractility in this study was $LVdP/dt_{max}$, a parameter known to be influenced

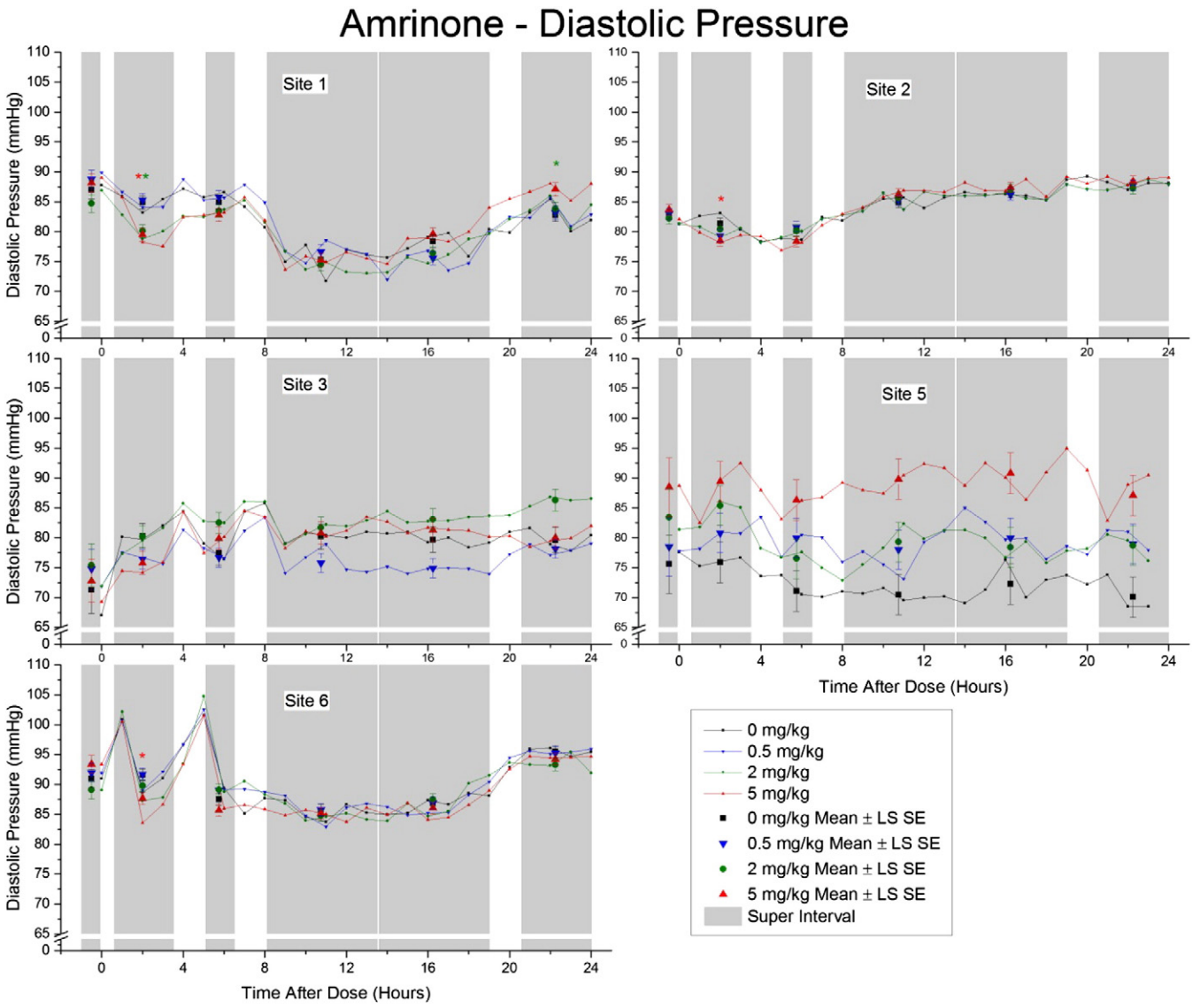

Fig. 10. Effect of amrinone on diastolic arterial blood pressure in conscious instrumented dogs at five different laboratories. Amrinone (vehicle = □, 0.5 mg/kg = ∇, 2 mg/kg = •, 5 mg/kg = Δ) administered orally at time = 0. Small symbols represent the mean value from the previous 10-minutes. Shaded areas represent super-intervals as defined in Table 3. Large symbols represent the mean ± SE within each super-interval.

indirectly by heart rate, ventricular preload (here estimated using LVEDP) and ventricular afterload (estimated using diastolic arterial pressure). The effect of the four test articles on these parameters is summarized here.

3.8. Positive inotropic drugs

3.8.1. Amrinone

The effect of amrinone on heart rate was not uniform across the five labs that tested it (Fig. 6). In three, there was no effect on heart rate at any dose or any time following administrations. In two laboratories, there was an increase in heart rate restricted only to the high dose treatment group and seen only in the first or second superinterval, thereby being transient in nature. In the three labs not finding a heart rate effect, there was a decrease in diastolic arterial blood pressure with the high dose (also with the mid dose in one lab), whereas it was unchanged in the two labs having the heart rate increase (Fig. 10). In only one of the labs, a decrease in LVEDP was seen following the high dose (Fig. 14). In all other labs, no effects on LVEDP were noted.

3.8.2. Pimobendan

Pimobendan administration was associated with only minor hemodynamic effects. Heart rate was not affected in two of three labs, with a slight increase following the high dose in one lab (Fig. 7). With the high dose there was also a small decrease in diastolic arterial pressure, which was also seen with the mid dose in one lab (Fig. 11). LVEDP was reduced in two labs with both mid and high doses during the first and second super-intervals, in one lab no significant effect was detected (Fig. 15).

3.9. Negative inotropic drugs

3.9.1. Atenolol

The hemodynamic response to atenolol was generally mild. None of the laboratories detected an effect on the LVEDP at any time or dose (Fig. 16). Two of three labs also found no effect on heart rate, whereas one found a significant decrease with each of the three doses tested (Fig. 8). Systemic diastolic arterial blood pressure was reduced following the mid and high doses in two of the studies but was unchanged in the third (Fig. 12).

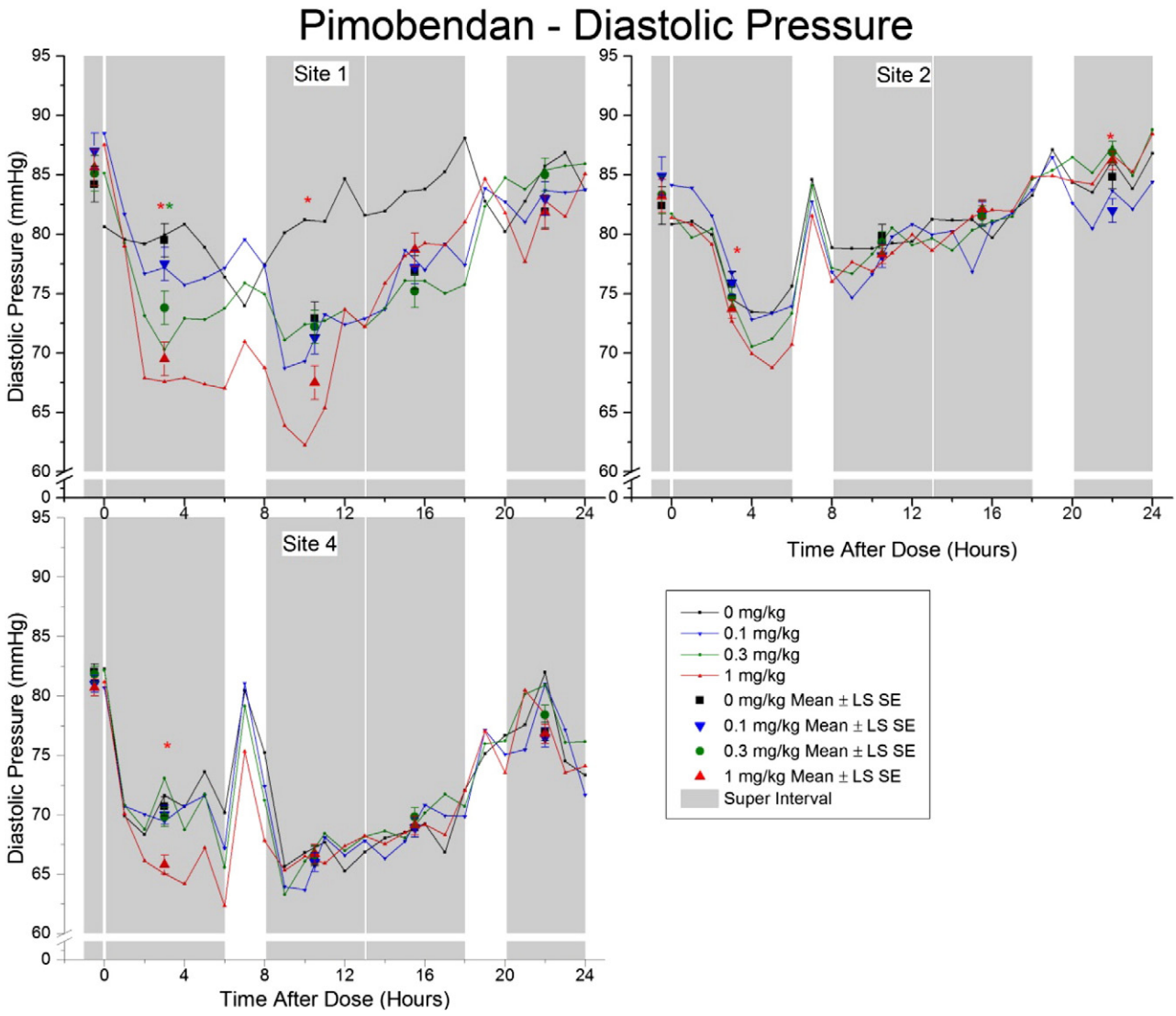

Fig. 11. Effect of pimobendan on diastolic arterial blood pressure in conscious instrumented dogs at three different laboratories. Pimobendan (vehicle = □, 0.1 mg/kg = ∇, 0.3 mg/kg = •, 1 mg/kg = Δ) administered orally at time = 0. Small symbols represent the mean value from the previous 10-minutes. Shaded areas represent super-intervals as defined in Table 3. Large symbols represent the mean ± SE within each super-interval.

3.9.2. Itraconazole

Itraconazole had little effect on LVEDP in all of the four studies (Fig. 17). Systemic diastolic arterial pressure was either not affected, or only mildly increasing in one study and decreasing in another following the high dose (Fig. 13). Heart rate tended to also be unchanged although one study had an increase following only the high dose (Fig. 9).

4. Discussion

The purpose of this study was to determine if a commonly used conscious dog model is capable of detecting changes in myocardial contractility when used for assessing drug-induced effects on the cardiovascular system. This consortium was proposed based on the results from a HESI supported workshop aimed to identify issues and opportunities for improving our preclinical cardiovascular profiling of new drugs (Sarazan et al., 2011). A recommendation coming from that workshop was that drug-induced effects on myocardial contractility should be evaluated routinely and it was suggested that non-clinical models are available to conduct such assessments. However, up to now the robustness of such models was never objectively evaluated, particularly including the potential for cross-laboratory variability. The present study has demonstrated that very similar experimental results could be obtained from the different laboratories participating in this consortium. All labs correctly categorized their assigned test compounds as being either a positive or negative inotropic agent at the doses tested. Moreover, the effects were consistently shown to be dose-dependent and reversible, further demonstrating the comparability of the effects detected.

A second issue of interest, with regards to the translation of such preclinical data to the clinic, involves the doses, or more importantly the achieved plasma drug concentrations, associated with a given effect on myocardial contractility, i.e., PK/PD. In the best case, the preclinical model should respond to the drug-induced effect on contractility at drug concentrations similar to those known to have similar effects in the clinical setting. Clearly, if the preclinical models are less sensitive to the clinical setting, one might either miss or underestimate the importance of an effect. Conversely, should the preclinical model be more sensitive to the effects than what is seen clinically, the model may incorrectly overestimate the risk for clinically relevant effects and could therefore lead erroneously to the discontinuation of the development of a safe drug. As mentioned below for the individual drugs tested, the relationship between the drug concentrations shown to cause dose-dependent

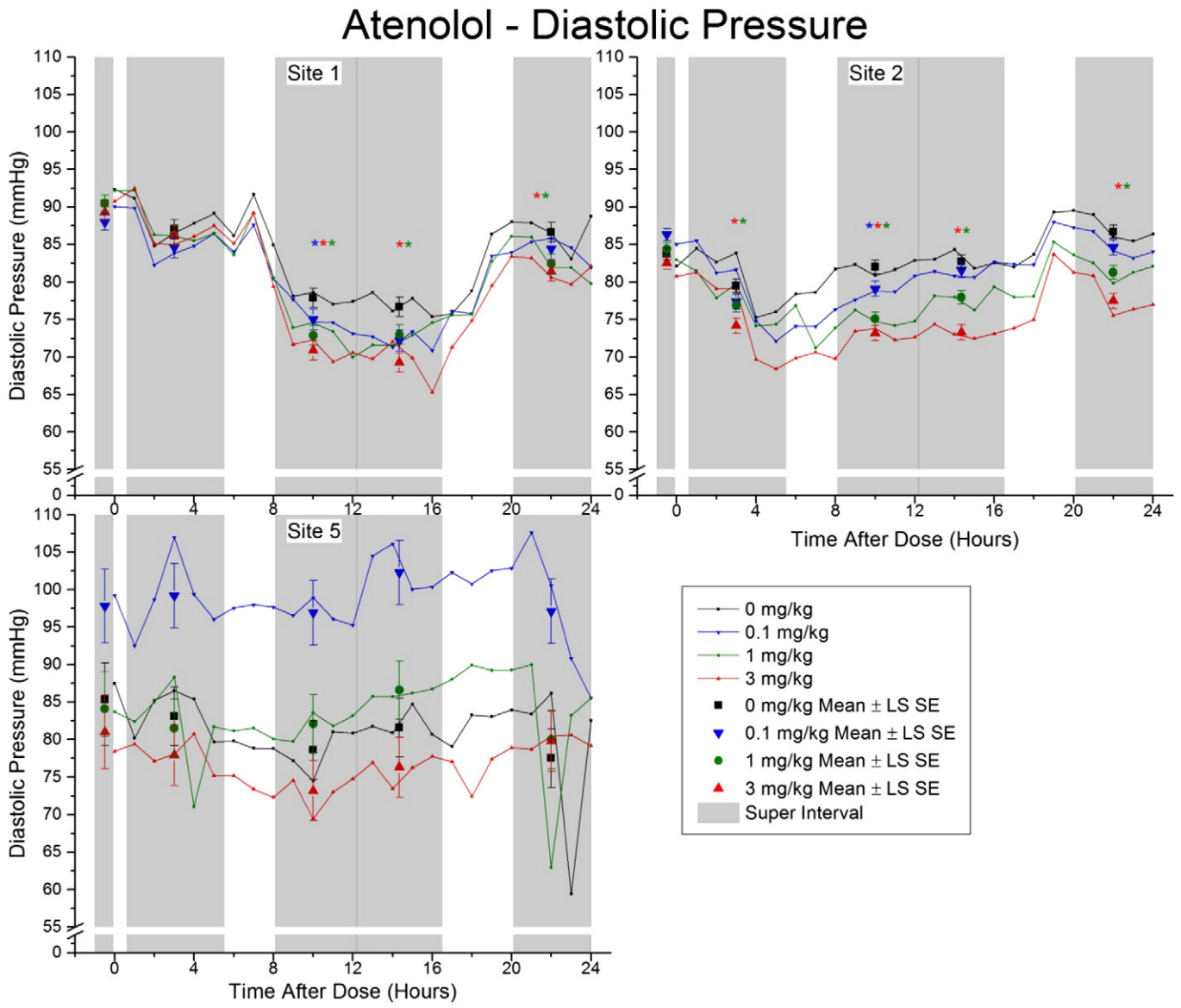

Fig. 12. Effect of atenolol on diastolic arterial blood pressure in conscious instrumented dogs at three different laboratories. Atenolol (vehicle = □, 0.1 mg/kg = ▽, 0.3 mg/kg = •, 1 mg/kg = Δ) administered orally at time = 0. Small symbols represent the mean value from the previous 10-minutes. Shaded areas represent super-intervals as defined in Table 3. Large symbols represent the mean ± SE within each super-interval.

effects in the present dog model appear to be remarkably close to literature data on doses and plasma drug concentrations found to cause the effects clinically. One must acknowledge the difficulty in comparing preclinical and clinical data to the inherent difference between these study types and the types of measurements used. Nevertheless, the correlation between the effect on myocardial contractility to drug concentration in both preclinical and clinical settings appears to be quite good. This underscores the importance of including measurements of plasma drug levels in preclinical studies. Whereas taking blood samples in the conscious animals is associated with a temporary disturbance of the hemodynamic measurements being made, in well-trained animals the effects are brief and quickly reversible. Furthermore, use of super-intervals in the data evaluation (see below) can be used effectively to eliminate transient artifacts in the data set by simply excluding times during which the animals are excited due to either feeding or the drawing of blood. Given the importance of the measurement of drug levels for the interpretation of the data, taking a single blood sample at some time shortly after the expected t_{max} appears to be a good compromise.

A further area of concern in this consortium study was the selection of $LVdP/dt_{max}$ as the primary index of contractile function. From theoretical considerations, based on the possible influences of ventricular preload and afterload on the assessment parameter, looking for changes in inotropic state by using the pressure–volume relationship throughout the cardiac cycle may be preferable (Suga et al., 1973). Its use in the conscious, freely moving dog is technically less feasible, such that the consortium selected the more commonly used and more accessible parameter of $LVdP/dt_{max}$, the maximal rate of pressure development in the left ventricle during systole. It was proposed that large changes in heart rate and ventricular preload or afterload that could potentially affect the use of $LVdP/dt_{max}$ as a contractility index, would not be expected in the context of a drug development, as such large effects would be viewed critically, independently from possible changes in myocardial contractility. Furthermore, with the continuous measurement of heart rate, left ventricular pressure and arterial blood pressure, the experimental model monitors the parameters that can have an impact on $LVdP/dt_{max}$. Thus, changes in $LVdP/dt_{max}$ devoid of relevant changes in these parameters can be assumed to reflect real, drug-induced changes

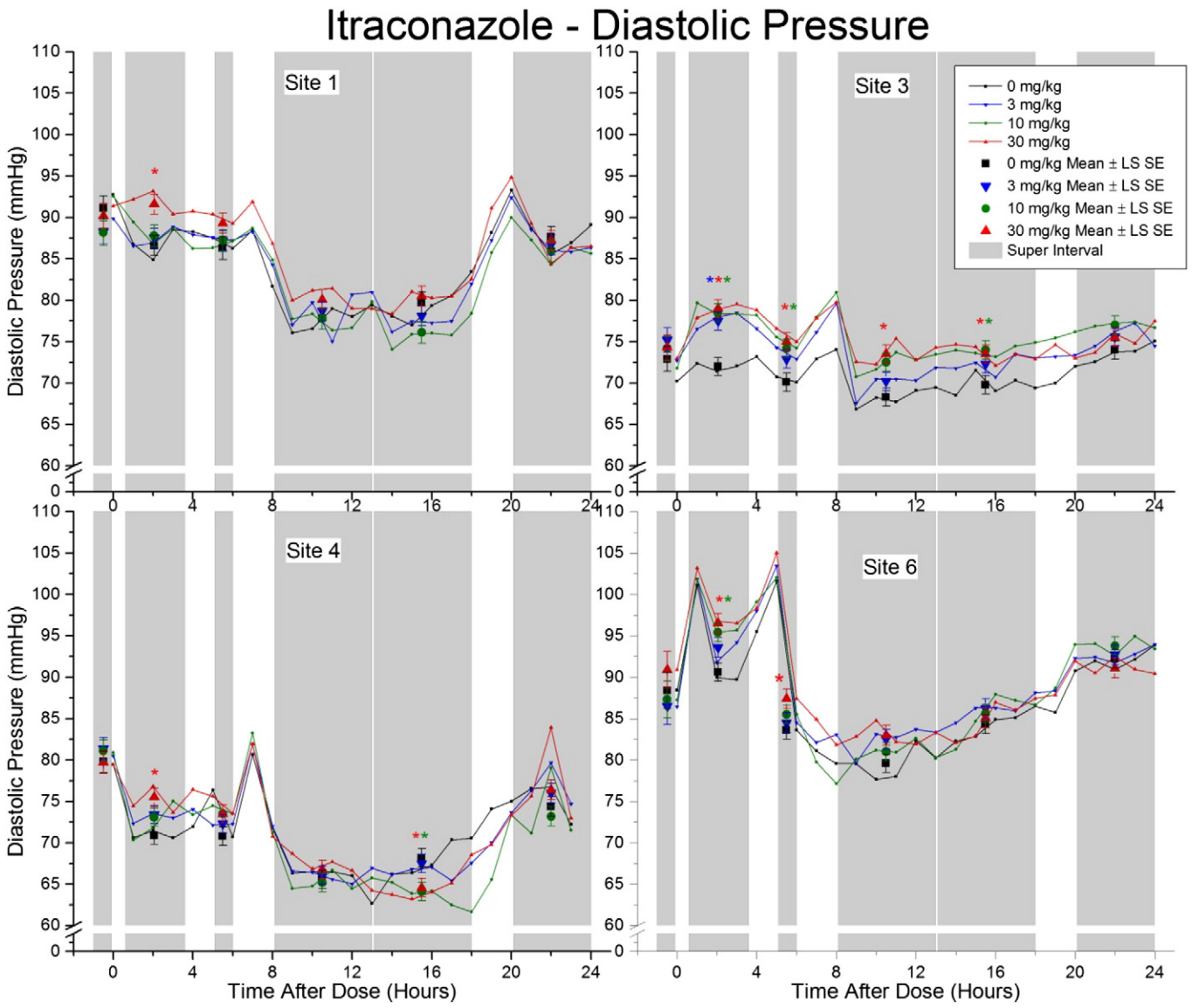

Fig. 13. Effect of itraconazole on diastolic arterial blood pressure in conscious instrumented dogs at four different laboratories. Itraconazole (vehicle = □, 3 mg/kg = ∇, 10 mg/kg = •, 30 mg/kg = Δ) administered orally at time = 0. Small symbols represent the mean value from the previous 10-minutes. Shaded areas represent super-intervals as defined in Table 3. Large symbols represent the mean ± SE within each super-interval.

in the inotropic state of the heart. In the present study, the effect of the four test compounds on heart rate, end-diastolic left ventricular pressure and mean arterial pressure were, in fact, minimal. Only with pimobendan was there a consistent effect on LVEDP, the effect being a decrease following the mid and high dose in two of three labs. Since a decrease in LVEDP would lead to a decrease in $LVdP/dt_{max}$, based upon the Cyon–Frank–Starling effect (see Zimmer, 2001), this obviously did not have a relevant impact on the observed increase in $LVdP/dt_{max}$. Diastolic aortic pressure was found to be reduced in 3 of 5 labs testing amrinone, an effect seen primarily with the high dose. Such a decrease would be expected to reduce $LVdP/dt_{max}$ and would counteract the observed increase in $LVdP/dt_{max}$ indicating that this change was not relevant for the detection of the positive inotropic effect. Slight reduction in diastolic aortic pressure was found with pimobendan (all three labs) as well as with atenolol (2 of three labs with both mid and high doses). As with amrinone, the observed decrease in diastolic aortic pressure would tend to minimize the observed increase in $LVdP/dt_{max}$ and can be interpreted to have little relevance for the inotropic assessment. The decrease in diastolic aortic pressure observed with atenolol is likely a result of the negative inotropic effect, but it must be recognized that it could also play a role in the reduced $LVdP/dt_{max}$ as well.

$LVdP/dt_{max}$ is dependent on heart rate, being based physiologically on the force–frequency relationship of the heart (Markert et al., 2007) and is therefore a further source of potential error when using this parameter to assess changes in contractile state of the heart. Except for slight increases in heart rate with the highest doses of amrinone and pimobendan tested, most compounds and doses were not associated with marked changes in the heart rate suggesting that the $LVdP/dt_{max}$ is correctly reflecting changes in myocardial contractility. With amrinone, 2 of 5 labs observed an increase in heart rate following the high dose, meaning that part of the observed increase in $LVdP/dt_{max}$ may be heart rate-dependent. Taken together, the changes observed with drug administration on parameters reflecting the preload and afterload of the left ventricle were modest and in some cases would have minimized the change in $LVdP/dt_{max}$ anticipated for the inotropic change. Thus, the results of this study support the use of $LVdP/dt_{max}$ as an index of left ventricular contractility in the context of testing for cardiovascular effects of drug candidates.

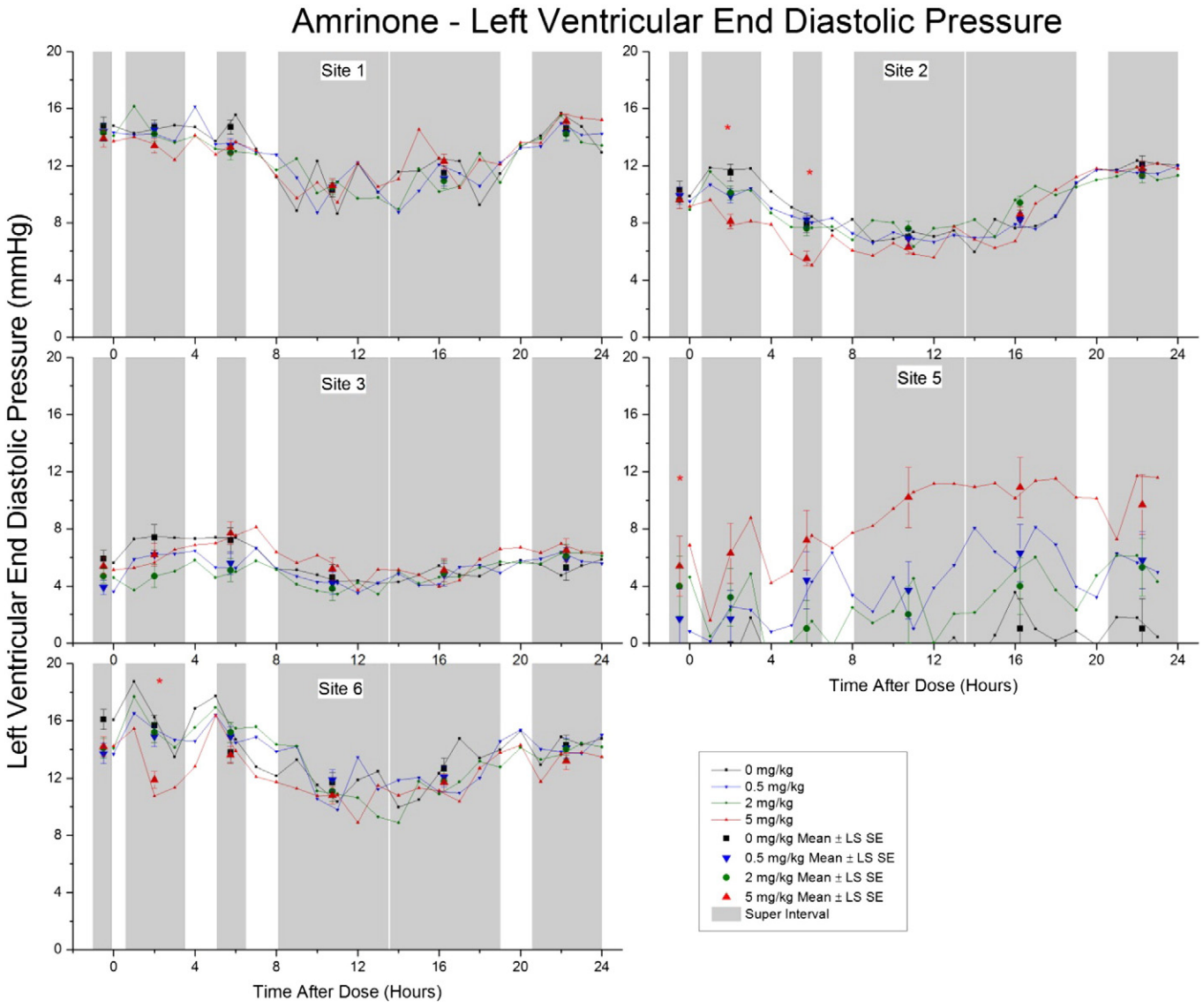

Fig. 14. Effect of amrinone on left ventricular end-diastolic pressure in conscious instrumented dogs at five different laboratories. Amrinone (vehicle = □, 0.5 mg/kg = ∇, 2 mg/kg = •, 5 mg/kg = Δ) administered orally at time = 0. Small symbols represent the mean value from the previous 10-minutes. Shaded areas represent super-intervals as defined in Table 3. Large symbols represent the mean ± SE within each super-interval.

There are other experimental approaches to the assessment of drug-induced effects on the inotropic state of the heart. Indeed, in the context of this consortium study, several other parameters were evaluated that may have value in detecting and quantifying changes in myocardial contractility. Some are based on hemodynamic data, whereas others employ different technologies, such as echocardiography. A comparison of these other approaches, in comparison to the results of the present study will be the subject of subsequent publications.

The members of the consortium selected four compounds to be used in this study, two expected to have a positive inotropic effect based on clinical experience with the drug and two expected to have a negative inotropic effect. Preference was given to drugs that were thought to have rather selective effects on myocardial contractility without simultaneous effects on arterial blood pressure or heart rate to reduce the role of secondary effects in the assessment. This goal was achieved overall, as discussed above. A further consideration was the availability of clinical data demonstrating the relationship of the inotropic effect to dose or plasma drug levels, to allow for a comparison of the data generated in the present study. Ultimately, pimobendan and amrinone were selected as the two positive inotropic agents and atenolol and itraconazole were selected as the negative inotropic agents.

In the present study using the chronically instrumented Beagle dog, all participating laboratories were able to demonstrate the anticipated effect on $LVdP/dt_{max}$ of the compounds tested, thereby classifying them correctly as either positive or negative inotropic agents. The compound-induced effects observed could furthermore be shown to be dose-dependent and reversible, two characteristics that also support the conclusion that the effects seen were drug-dependent. Efforts were made to eliminate methodological differences between the participating laboratories, but some differences that may have affected the outcome remained. Nevertheless, the overall results between laboratories were very similar, particularly when drug-induced effects were assessed as a change from the respective baseline values (see below for more discussion). Furthermore, the plasma concentrations found to produce positive or negative inotropic effects in the dog were very similar to the plasma drug concentrations known to be inotropic in man. At least based on these four drugs, there seems to be an excellent correlation between the exposure–response relationship in the dog as

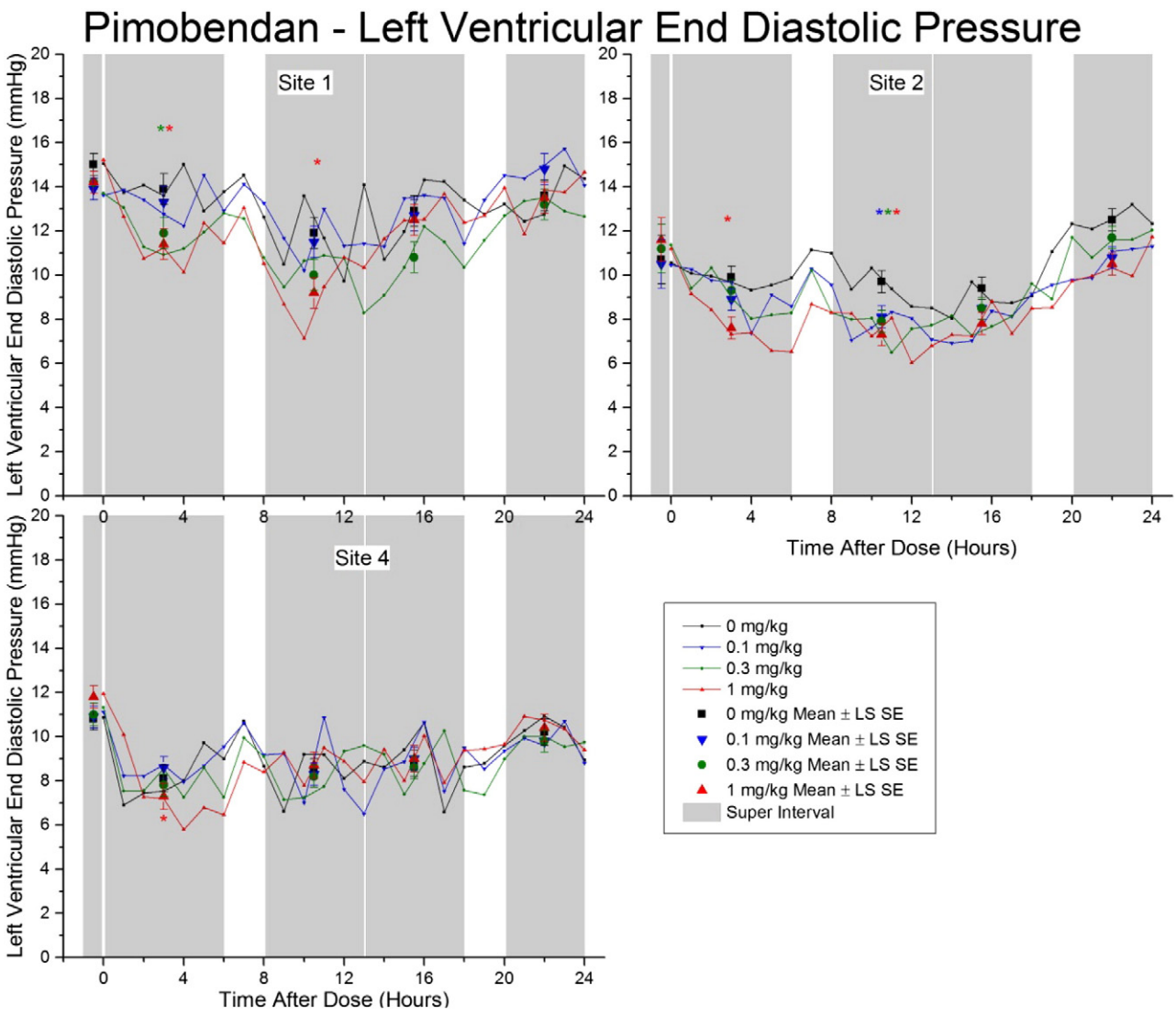

Fig. 15. Effect of pimobendan on ventricular end-diastolic pressure in conscious instrumented dogs at three different laboratories. Pimobendan (vehicle = □, 0.1 mg/kg = ∇, 0.3 mg/kg = •, 1 mg/kg = Δ) administered orally at time = 0. Small symbols represent the mean value from the previous 10-minutes. Shaded areas represent super-intervals as defined in Table 3. Large symbols represent the mean ± SE within each super-interval.

compared to man. As such, the conscious dog model used in these studies appears well suited for testing new drugs for potential inotropic effects.

4.1. Amrinone

Amrinone is a bipyridine phosphodiesterase III inhibitor known to increase myocardial contractility and to be a vasodilator (Alousi & Johnson, 1986). It is used for short-term treatment of severe heart failure based on its positive inotropic action as well as its reduction of arterial blood pressure (afterload), both effects leading to an increase in cardiac output. In the present dog study, the oral doses of 0.5, 2 and 5 mg/kg produced modest, dose-dependent increases in $LVdP/dt_{max}$. In the pharmacokinetic study conducted in the healthy Beagle dog, the maximal plasma concentrations of amrinone following the three oral doses used in this study were 269, 743 and 2652 ng/mL. In man, amrinone has been reported to have a positive effect on left ventricular function at a plasma concentration of 1200 ng/mL (Kullberg, Freeman, Biddlecome, Alousi, & Edleson, 1981; Ward, Brogden, Heel, Speight, & Avery, 1983) thereby being similar to the mid and high doses used in the present study; supporting a good correlation between the plasma drug levels causing an increase in myocardial contractility in the healthy dog versus that in man.

4.2. Pimobendan

Pimobendan was an interesting compound for this study, as it causes a rather selective positive inotropic action on the heart with little effect on heart rate, at least in lower doses. It was developed clinically for the treatment of heart failure but reached the market only in Japan (Kato, 1997). Nevertheless, it is a mainstay for the treatment of heart failure in veterinary medicine, most notably in dogs (Atkins, Bonagura, Ettinger, et al., 2009). Its positive inotropic action is described to be due to both its phosphodiesterase III inhibition as well as its calcium sensitizing properties (Van Meel & Diederen, 1989). Pimobendan was shown to have a positive inotropic effect in healthy volunteers using echocardiography when treated with an oral dose of 7.5 mg (Chu, Hu, & Shieh, 1999). Fractional shortening was increased by 46% and ejection fraction was increased by 29.8% with a maximal plasma concentration (total of both enantiomeres) of about 13 ng/mL. It should be noted that in this study with the oral dose of 7.5 mg there was also a significant increase in heart rate (38%) suggesting that this dose was larger than that needed

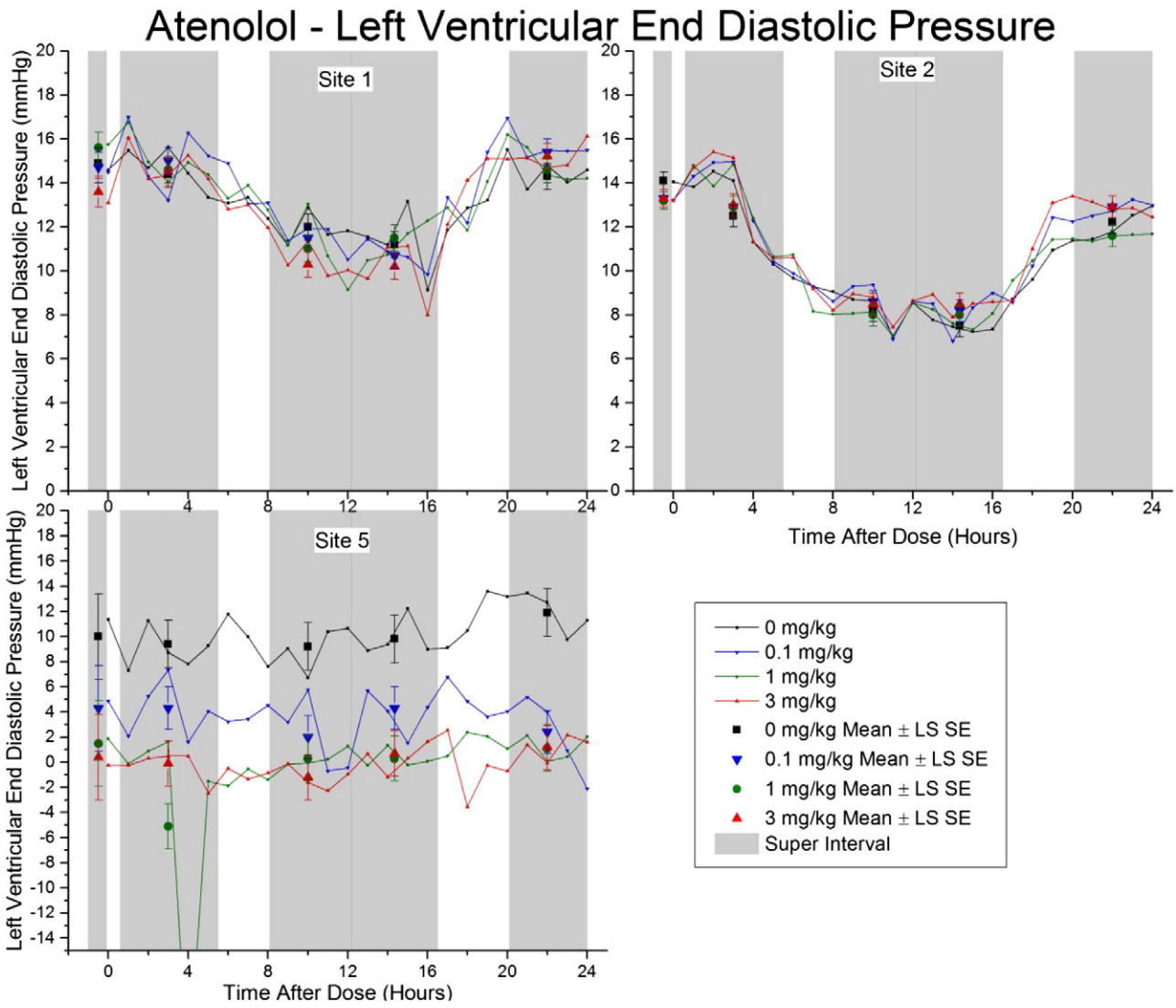

Fig. 16. Effect of atenolol on ventricular end-diastolic pressure in conscious instrumented dogs at three different laboratories. Atenolol (vehicle = □, 0.1 mg/kg = ∇, 0.3 mg/kg = •, 1 mg/kg = Δ) administered orally at time = 0. Small symbols represent the mean value from the previous 10-minutes. Shaded areas represent super-intervals as defined in Table 3. Large symbols represent the mean ± SE within each super-interval.

to elicit a more selective effect on the inotropic state. In the present dog study, a dose-dependent increase of $LVdP/dt_{max}$ was observed across laboratories starting with the low dose of 0.1 mg/kg and increasing dose-dependently up to the high dose of 1 mg/kg which was associated with a C_{max} of 22.9 ng/mL in the independent pharmacokinetic study. With the high dose used in the dog, there was also an increase in heart rate. Thus, the plasma concentrations of pimobendan shown to be positive inotropic in the healthy Beagle dog correspond very well to the plasma drug levels shown to be positive inotropic in the healthy human volunteer.

4.3. Atenolol

As a selective cardiac ß$_1$-adrenoceptor blocker, atenolol is expected to reduce myocardial contractility and heart rate, particularly in hearts under ß-adrenoceptor activation through neural or humoral stimulation. It is widely used clinically for the treatment of hypertension, angina and certain arrhythmias. It was shown to produce negative inotropic effects in the human heart at plasma concentrations of 330 ng/mL (de Abreu, de Castro, & Pedrazzoli, 2003; Thomas et al., 1992). In the present dog study oral doses of 0.3, 1 or 3 mg/kg were tested and found to reduce $LVdP/dt_{max}$ dose-dependently. In the independent pharmacokinetic study conducted in the Beagle dog, the C_{max} values for these three oral doses of atenolol were 174, 538 and 1718 ng/mL, respectively, demonstrating a very similar range of plasma drug concentrations to those known to cause negative inotropic effects in man.

4.4. Itraconazole

Itraconazole (Sporanox®) is a synthetic triazole antifungal agent that was selected for this study on the basis of its causing negative inotropic effects with its clinical use, which ultimately led to a boxed warning in its prescribing information. It has been shown to be associated with heart failure with its clinical use, despite its negative inotropic activity going undetected in both preclinical and clinical trials (Slordal & Spigset, 2006). As such, it is a prime candidate for use in this study, posing the question whether or not the use of this experimental model would have detected this effect, had such studies been performed during the development of the compound. It has been reported that itraconazole has a negative effect on myocardial contractility in humans at plasma drug concentrations of about 1000 ng/mL (Ahmad, Singer, & Leiss, 2001; Hardin et al., 1988). In the pharmacokinetic study conducted as a part of the present evaluation in Beagle dogs, the oral doses of itraconazole used (3, 10 and 30 mg/kg) produced C_{max} values of 373,

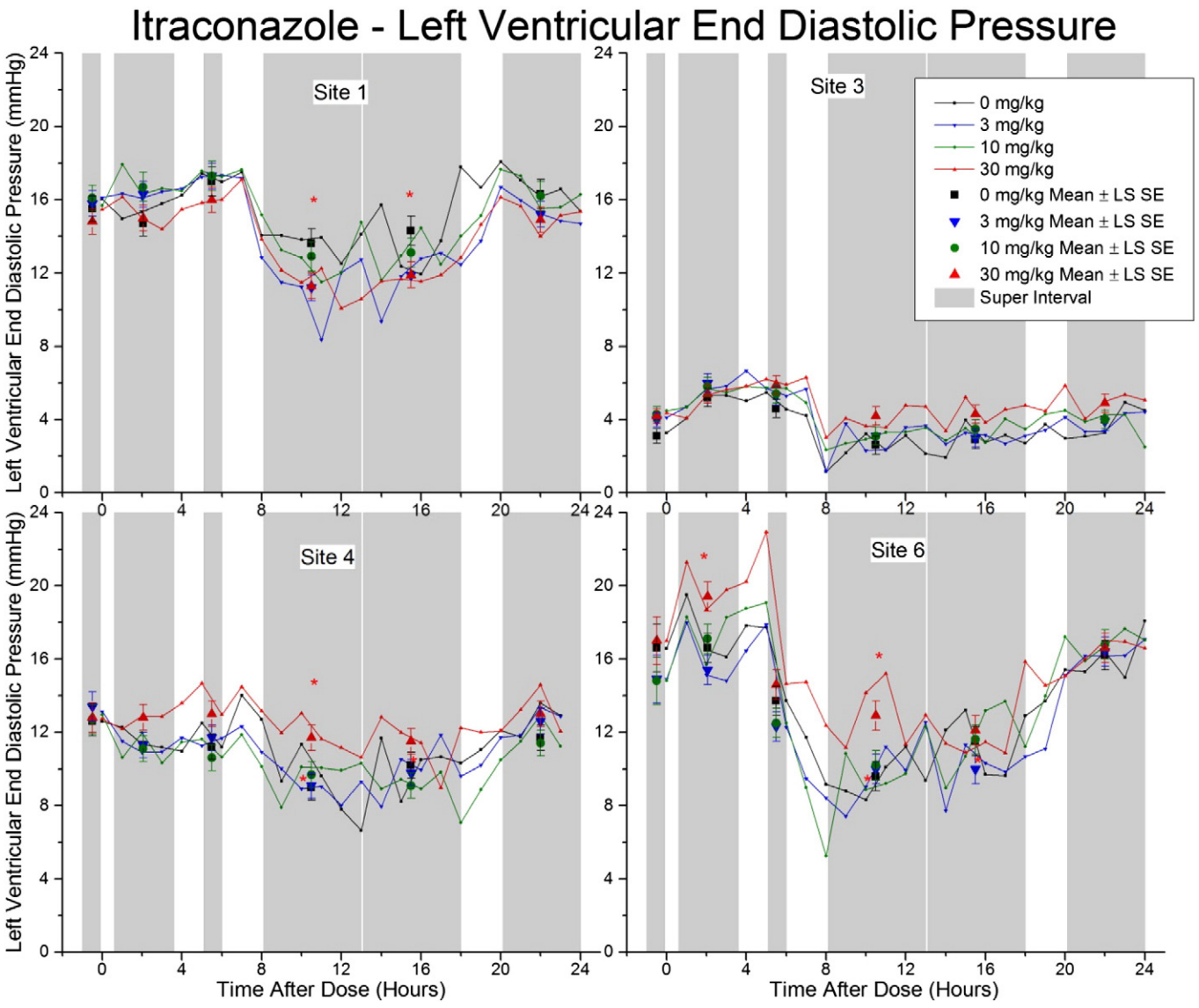

Fig. 17. Effect of itraconazole on ventricular end-diastolic pressure in conscious instrumented dogs at four different laboratories. Itraconazole (vehicle = □, 3 mg/kg = ∇, 10 mg/kg = •, 30 mg/kg = Δ) administered orally at time = 0. Small symbols represent the mean value from the previous 10-minutes. Shaded areas represent super-intervals as defined in Table 3. Large symbols represent the mean ± SE within each super-interval.

1253 or 2127 ng/mL respectively, suggesting that both mid (10 mg/kg) and high doses (30 mg/kg) should have been associated with a negative inotropic effect, provided that there is a good correlation between the clinical observations and those in dogs. In fact, the mid and high doses of itraconazole in this study decreased inotropy in the dog at similar plasma concentrations to those detected in man.

Taken together, with all four test compounds there seems to be a remarkably good correlation between plasma drug concentrations found to affect $LVdP/dt_{max}$ and those reported to cause inotropic effects in man.

Qu et al. (2013) have recently published a study aimed to define the mechanism of action for the negative inotropic effects of itraconazole (Qu et al., 2013). Despite testing itraconazole in a variety of both in vitro and in vivo models, these investigators could not determine the responsible mechanism of action. They did manage to demonstrate that it is a direct effect on the heart since it can be seen in isolated heart preparations, and they have eliminated L-type Ca^{++} blockade or involvement of the mitochondria as possible mechanisms.

The data generated in these dog studies demonstrate that the conscious dog model can detect both positive and negative inotropic activity. The conscious dog is already one of the most commonly used experimental models for testing drugs for their potential cardiovascular effects. The addition of a high fidelity micromanometer into the left ventricle of this animal species in this model allows for the detection of inotropic effects in addition to heart rate, arterial blood pressure and the ECG. Despite the best efforts of the participants, it was not possible to harmonize all aspects of the study design in order to conduct similarly. Nevertheless, the comparability of the overall outcomes is noteworthy. Some differences between the participating sites were noted, however. In particular, the baseline values of some of the parameters measured were not uniform. This is perhaps best illustrated by the baseline heart rate values reported, that ranged between studies across laboratories testing amrinone and from a low, group baseline value of 85.6 beats per minute to a high, group baseline value of 133.3 beats per minute. Whereas environmental factors can influence the baseline hemodynamic values obtained, it was also reported that the dogs having the high HR values at baseline were relatively young and had been recently instrumented. As such, they were less acclimatized to the study environment and were likely more excitable than in older, more thoroughly trained animals used in some of the other laboratories. It should be emphasized,

however, that despite the difference in baseline values, the changes in inotropic state, as assessed using $LVdP/dt_{max}$ calculated as a change from the baseline and compared to treatment with the vehicle, could be clearly demonstrated in all laboratories, independently from their baseline levels.

4.5. Data evaluation

A plot of the mean values of each parameter measured over time, using short (e.g. 5 min) intervals to average the data, provides a valuable overview of possible drug-induced effects. At a glance one can assess the dose-dependency of any effect detected, the time of its onset, and the degree of reversibility over time. Since data are accumulated continuously over time using the telemetry-based systems, there are a variety of data analysis approaches dependent upon how one chooses to subdivide the data over time. For graphic representation, most laboratories have used a 5–10 min mean value calculation over the 24 h of data collection. This type of analysis gives a rather high level of detail for time-dependent effects but can have a higher degree of variability merely based on the short time intervals. The statistical analysis of this type of data set can also lead to spurious effects for a given point in time. Therefore, for statistical analysis, larger time intervals are typically selected, extending to the extreme case of defining super-intervals that can extend for over an hour or more, and are selected to compare possible drug-induced effects over the time period during which the effects appear to be largest by visual inspection (Sivarajah et al., 2010). In most cases one would anticipate the largest effects to be associated with the time of maximal drug concentration t_{max}. However, there are situations in which there may not be a direct correlation between the plasma levels of a given test article and the resultant hemodynamic effects seen, such as with cases where metabolites may play an important role in hemodynamic effects observed. In these cases, the defining of super-intervals based on the observed effects, as opposed to the timing of the plasma drug levels is advantageous in describing the drug-induced effects. Finally, as mentioned above, defining the super-intervals to exclude times in which the animals are excited for reasons other than drug-induced effects can be used effectively in the data evaluation. For example, in the studies conducted with amrinone the animals had blood drawn 4 h post-dosing and were fed at 7 h post-dosing. The super-intervals selected for evaluation (0.5–3.5, 5–6.5 and 8–13.5 h post-dosing) specifically excluded these times thereby eliminating these potential artifacts from the analysis.

5. Conclusion

In this first report coming from a HESI-based consortium focusing on drug-induced effects on myocardial contractility, $LVdP/dt_{max}$ was shown to be a useful parameter to detect effects in the conscious, chronically instrumented dog across 6 of the participating laboratories. Using this experimental approach, all laboratories could identify the inotropic effect of the test agents and these effects could be correlated to the plasma drug levels measured in the same studies. These, in turn, appear to be very similar to the plasma drug levels in man shown to have inotropic effects with their clinical use, thereby establishing a good translation between the preclinical and clinical settings.

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Disclaimer

The opinions presented here are those of the authors. No official support or endorsement by US Food and Drug Administration is intended or should be inferred.

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