Orally Available Levosimendan:

Dose-Related Positive Inotropic and Lusitropic Effect in

Conscious, Chronically-Instrumented Normal and Heart Failure Dogs

Satoshi Masutani
Heng-Jie Cheng
Minja Hyttilä-Hopponen
Jouko Levijoki
Aira Heikkilä
Arja Vuorela
William C Little
Che-Ping Cheng

The primary laboratory of origin: Wake Forest University School of Medicine

Winston-Salem, NC (S.M., H.J.C., W.C.L., C.P.C.)

Affiliation: Orion Pharma, Espoo, Finland (M.H.H., J.L., A.H., A.V.)
Running title page:

a) Oral Levosimendan Improves Cardiac Performance in HF

b) Address correspondence to: Che-Ping Cheng, MD, PhD,
   Cardiology Section, Wake Forest University School of Medicine,
   Medical Center Boulevard, Winston-Salem, North Carolina 27157-1045.
   E-mail: ccheng@wfubmc.edu

c) The number of text pages: 25
   The number of tables: 3
   The number of figures: 5
   The number of references: 36
   The number of words in the Abstract: 248
   The number of words in the Introduction: 543
   The number of words in the Discussion: 1,253

d) A list of nonstandard abbreviations used in the paper.
   LS, levosimendan; HF, heart failure; LV, left ventricular

e) A recommended section assignment to guide the listing in the table of contents.
   Section options are: Cardiovascular
Abstract

Levosimendan (LS), a Ca^{2+} sensitizer, is presently limited to intravenous administration. The dose-related pharmacodynamic effects of newly-developed oral LS remain undetermined. We assessed the dose-response relationship of oral LS in nine normal and seven pacing-induced heart failure (HF), conscious, chronically-instrumented mongrel dogs. Animals received a placebo capsule on day 1, and then LS was administered at single oral doses of 0.025 (day2), 0.05 (day4) and 0.1 (day8) mg/kg. We serially measured plasma LS concentrations, hemodynamic, and left ventricular (LV) systolic and diastolic functional responses periodically until 12 h after oral LS. In both normal and HF, after 3 incremental dosages of oral LS, the peak plasma LS concentrations (34.6, 66.8, 123.2 in normal; 38.3, 71.5, 137.4 ng/ml in HF) were achieved within 2 h in proportion to the dose, parallel to an increased LV contractility (E_{ES}) (normal: from 5.7 of placebo to 8.2, 10.5 and 12.6; HF: from 3.7 of placebo to 5.7, 7.1 and 8.7 mmHg/ml), and decreased time constant of LV relaxation (τ) (normal: from 28.8 of placebo to 25.6, 24.7, and 23.5; HF: from 44.7 of placebo to 38.9, 36.4 and 34.6 msec). Compared with placebo, total systemic vascular resistance and mean left atrial pressure were significantly reduced after LS. In HF, oral LS caused a dose-dependent increase of LV-arterial coupling and mechanical efficiency. Heart rate increased only after 0.1 mg/kg of LS in normal dogs. In conclusion, oral LS produces vasodilatation and dose-dependent augmentation in LV contractility and relaxation both in normal and HF.
Introduction

Despite enormous advances in the understanding and treatment of heart failure (HF) that have taken place over the past 50 years, HF remains a leading cause of morbidity and mortality worldwide. Although depressed pump function is common, the clinical use of effective inotropic therapy to safely stimulate contraction has been difficult. (Kass and Solaro, 2006; Lehtonen and Poder, 2007) Conventional inotropic agents (e.g., β-adrenergic agonists, dobutamine and phosphodiesterase inhibitors, milrinone) increase myocardial contractility by enhancing cAMP and protein kinase A (PKA) to stimulate the activation of Ca\(^{2+}\). Thus, the risk of Ca\(^{2+}\) overloading and arrhythmia is intrinsic to the mechanism of action. This approach has proven less effective in failing hearts because of downregulation of the signaling and is chronically linked to toxicity and increased mortality. (Kass and Solaro, 2006)

An alternative is Levosimendan (LS), a novel Ca\(^{2+}\) sensitizer that acts very differently on the heart muscle, augmenting contractility by enhancing the calcium sensitivity of the contractile proteins and increasing the affinity of troponin C for Ca\(^{2+}\). (Pagel, et al., 1997; Harkin, et al., 1995); whereas, at higher concentrations, its action as a phosphodiesterase (PDE III) inhibitor contributes to the positive inotropic effect (Edes, et al., 1995; Harkin, et al., 1995; Kass and Solaro, 2006). LS also leads to peripheral and coronary vasodilation through the opening of ATP-sensitive potassium channels. (Yokoshiki, et al., 1997b) LS not only produces a dose-dependent increase in LV contractility, but also improves LV relaxation at rest and during exercise in normal and HF. (Tachibana, et al., 2005) Increased experimental studies and clinical data have demonstrated that the combination of K-channel opening with calcium sensitization
offers unique benefits in comparison to currently-available inotropes. (Lehtonen and Poder, 2007; Endoh, 2007) In contrast to dobutamine, LS does not increase myocardial oxygen demand and has anti-ischemic and anti-arrhythmic actions. (Ukkonen, et al., 2000; Pagel, et al., 1997; Avgeropoulou, et al., 2005; Moiseyev, et al., 2002) In patients with severe HF, LS exerts anti-inflammatory and anti-apoptotic properties. (Parissis, et al., 2004; Trikas, et al., 2006; Follath, et al., 2002) Compared with dobutamine, LS demonstrated superior hemodynamic improvement and safety for treatment of acute, decompensated HF (Follath, et al., 2002) and myocardial infarction complicated by LV dysfunction and HF. Although these are important advances for LS in HF therapy, the current use of LS is limited to short-term intravenous administration. A newly-developed capsule formulation of LS enables oral administration, which could provide a convenient method of LS delivery, and may offer an improved therapeutic approach for both acute and chronic management of HF. Previous studies of oral LS primarily reported non-invasive assessment (Hosenpud and Group, 1999; Poder, et al., 2004; Poder, et al., 2003) and right heart catheterization data. (Harjola, et al., 1999) More precise contractility assessments have not been performed, and the direct effects of oral LS on LV systolic and diastolic performance before and after HF are unclear. The dose-related pharmacodynamic effect of oral LS remains undetermined.

Accordingly, this study was undertaken to assess the time- and dose-dependent effects of oral LS on hemodynamic response, LV systolic and diastolic functional performance, LV-arterial coupling, and cardiac mechanical efficiency in normal and HF states. Our findings suggest the importance of pursuing further studies with oral LS for the long-term management in HF patients. These data advance the new concept of orally-
administered chronic LS therapy for HF.

Methods

Instrumentation

This investigation was approved by the Institutional Animal Care and Use Committee and conforms to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Pub. No. 85-23, Revised 1985). Nine healthy, adult, heartworm-negative mongrel dogs (body weight 24-38 kg) were chronically instrumented to measure three LV internal dimensions, LV pressure (P), and left atrial (LA) P. Myocardial leads (Model 4312, Cardiac Pacemakers) were implanted within the myocardium of the right ventricle and right atrium, and the leads were attached to unipolar multi-programmable pacemakers (Model 8329, Medtronic, Minneapolis, MN) positioned under the skin in the chest. Hydraulic occluders were placed around the venae cavae by a technique previously described. (Cheng, et al., 1996; Tachibana, et al., 2005; Morimoto, et al., 2004)

Data Collection

Studies were performed after full recovery from instrumentation (two weeks after original surgery) with the animals standing quietly and unsedated in a sling. Data were acquired and analyzed using customized software (SPECTRUM v 5.0) as previously described. (Cheng, et al., 1996; Tachibana, et al., 2005)
Experimental Protocol

Studies before HF

To determine the dose-response relation of oral LS and assess the efficiency of oral LS in comparison with intravenous LS and dobutamine in normal dogs, the following interventions were studied in random order. The animals were equilibrated for 3 days between studies.

Effect of Oral LS. Following an 8-day study period, animals received 3 incremental dosages of oral LS (immediate-release, white, and hard-gelatin capsules of 0.25, 0.5, and 1 mg supplied by Pharmaceutical Product Development, Orion Pharma, Orion Corporation, Turku, Finland).

Early (8:00 am) on Day 1, before any medication and food intake, steady-state hemodynamic data were obtained with the animals unsedated and standing quietly on the sling. Variably loaded LV P-V loops were generated by sudden, transient occlusion of the vena cavae (VCO) as previously described. (Tachibana, et al., 2005) Then, a placebo capsule was given to the animals, and food intake was generally allowed after 1-2 hours following oral drug intake. Hemodynamic measurements were repeated at 0.5, 1, 1.5, 2, 3, 4, 6, 8, and 12 hours after placebo capsule administration.

Early (8:00 am) on Day 2, before any medication and food intake, initial steady-state and VCO data were obtained, and blood samples for plasma LS concentration measurements were collected. Then, a target dose of 0.025 mg/kg of LS was given to the animals. Hemodynamic measurements and blood sample collections were repeated at 0.5, 1, 1.5, 2, 3, 4, 6, 8, and 12 hours after oral administration of LS capsules.
Early (8:00 am) on Days 4 and 8, before any medication and food intake, after baseline hemodynamic data and blood sample collections, a target dose of 0.05 mg/kg (Day 4) and 0.1 mg/kg (Day 8) of LS was given to the animals, data were collected, and blood sampling was performed as described in Day 2.

**Effect of Intravenous LS.** After baseline hemodynamic data and blood sample collections, animals received intravenous LS (24 µg/kg over 10 min followed by 0.2 µg/kg/min for 40 min), and data and blood samples were collected again at 40 min. (Tachibana, et al., 2005)

**Effect of Intravenous Dobutamine.** After baseline hemodynamic data collections, animals received dobutamine intravenous infusion (2, 4, 6, 8 and 10 µg/kg/min at 10 min intervals). Data were again obtained.

**Induction of Stable HF**

Following the completion of normal studies, rapid right ventricular pacing was initiated. As previously described, (Cheng, et al., 2001) the pacing protocol producing a stable degree of HF was performed with rapid right ventricular pacing (210-240 bpm) for 3 weeks to induce HF followed by pacing at 190 bpm to maintain HF. To monitor the development of HF, the pacing was transiently discontinued twice per week, and steady-state and VCO data were recorded. When the LV end-diastolic P during the non-pacing period had increased by more than 15 mmHg over the pre-pacing control level and clinical signs of HF were seen, HF data were obtained.
Studies after HF

In all of the animals, LS studies started after the onset of HF plus one week of pacing at 190 bpm (i.e., during the stable HF period). Briefly, as described in our past report, (Cheng, et al., 2001) on the day of the experiment, the pacemaker was inactivated and the animals were allowed to equilibrate for one hour. The HF baseline hemodynamic data and control blood samples for oral and intravenous LS were collected. Then, oral LS (from Day 1 to Day 8), intravenous LS, and dobutamine administrations were repeated as described above for normal studies. The hemodynamic functional response and plasma LS concentrations in response to three incremental dosages of oral LS and intravenous LS were measured. Cardiac and hemodynamic responses to incremental dosages of dobutamine were obtained. Each day after the experiment, pacing was restarted at 190 bpm before animals returned to their rooms.

Data Processing and Analysis

As previously described, (Cheng, et al., 2001;Cheng, et al., 1996;Tachibana, et al., 2005) the LV volume (V) was calculated as a modified general ellipsoid. The rate of LV relaxation (τ), LV end-systolic P (PES)-end-systolic V (VES) relation and its slope (EES), and stroke work (SW)-end-diastolic V (VED) relation and its slope (MSW) were analyzed. To account for respiratory changes in intrathoracic P, steady-state measurements were averaged over the 12-second recording period spanning multiple respiratory cycles. The rate of LV relaxation (τ) was analyzed by determining the exponential time constant of the isovolumic fall of LV P by the Weiss method monoexponential decay model to zero. In addition, τ was also calculated by the non-zero asymptote. The mean end-systolic
circumference stress ($W_{ES}$, g/cm²) of LV was calculated by use of a thick-wall spherical model (Suga and Sagawa, 1979):

$$W_{ES} = G \cdot P_{ES} \cdot V_{ES}^{2/3} \cdot [(V_{ES} + Vm)^{2/3} - V_{ES}^{2/3}]^{-1}$$

Where $G (= 1.36)$ is a conversion factor from mmHg to g/cm², $P_{ES}$ is LV end-systolic P; $V_{ES}$ is LV end-systolic V. $V_m$ is the LV wall volume (ml), which is assumed to be 5 ml/kg body weight. (Suga and Sagawa, 1979) LV-arterial coupling was quantitated as the ratio of $E_{ES}$ to $E_A$, determined as $P_{ES}$/stroke volume (SV). LV P-V area (PVA), which represents the total mechanical energy, was determined as the area under end-systolic P-V relation and systolic P-V trajectory above $P_{ED}$-$V_{ED}$ curve. The efficiency of the conversion of mechanical energy to external work of the heart was calculated as SW/PVA. (Nozawa, et al., 1994)

**Determination of plasma LS concentrations**

One-milliliter blood samples were withdrawn from the peripheral venous catheter at each time point as described above and were placed into pre-cooled sterile Vacutainer tubes and centrifuged at 3,000 rpm for 10 min. The resulting plasma from each sample was stored at -80 °C for subsequent analysis. LS and its metabolite, OR-1855, as well as their internal standards, were separated from plasma using liquid-liquid extraction. The concentrations of LS and OR-1855 were determined by liquid chromatography tandem mass spectrometry (LC-MS/MS) at the Department of Pharmacokinetics and Bioanalytics, Orion Pharma, Orion Corporation using atmospheric pressure chemical ionization and detected using selected reaction monitoring. Internal standardization based on peak area
ratios was used for the quantification. The quantitation range of the method was 0.200-200 ng/ml for both analyses using 0.1 ml canine plasma.

**Statistical Analysis**

Data were summarized as mean ± SD (or indicated as mean±SEM). The peak hemodynamic effect time was determined by using key indexes, such as LV time constant, LV end-systolic volume, and contractility. Statistical comparisons were made with Student’s t-tests for paired observations. Indices of LV function and systemic hemodynamics were compared among the dosing groups by ANOVA for repeated measures. If the ANOVA revealed significant differences, individual group means were compared by use of Bonferroni procedure. Treatment effects were determined by ANCOVA on the outcome measures adjusted for baseline values. Repeated measured ANOVA was used for the time effect with separate paired t-tests in the presence of a significant interaction. Significance was established as \( p < 0.05 \). Curve fits on concentration-response plots were performed using sigmoidal dose-response assumption.

**Results**

A total of 9 animals were instrumented and fully recovered from surgery. Only seven animals successfully underwent induction of HF with the modified pacing protocol and had established stable HF. Thus, data are reported for 9 normal and 7 HF animals that had data recorded throughout the 8-day study period during oral LS administration. For the comparison between LS and dobutamine, data are reported only for the subgroup of 6
animals that had been collected with the 3 drug treatments (oral LS, intravenous LS, and
dobutamine) before and after HF.

Dose-Related Effects of Oral LS on Plasma LS Concentrations and Hemodynamic
and Cardiac Responses in Normal and HF

Over 8 days, both normal and HF animals received a placebo capsule on Day 1,
and then LS was administered at single oral target doses of 0.025 (LS1), 0.05 (LS2), and
0.1 mg/kg (LS3) on Day 2, Day 4, and Day 8, respectively. The target doses of oral LS
were absorbed and well tolerated without adverse events, such as arrhythmia or
hypotension.

Dose-Related Increases in Plasma LS Concentrations after Oral LS

As summarized in Table 1 and displayed in Figures 1 and 2A, in both normal and HF
animals, after 3 incremental dosages of oral LS, the plasma LS concentrations were
significantly elevated above 10 to 30 ng/ml within 0.5 to 2 hours. Similar peak plasma
concentrations of LS (C_max) were reached within 2 hours (T_max) in proportion to the dose
(Table 1). The similar pharmacokinetic parameters (such as C_max and T_max) of LS after
incremental dosages of oral LS administration showed that no accumulation of LS
occurred with any target dosing interval.

Figure 1 illustrates efficient plasma LS levels and concentration-response
relationship after oral LS in normal and HF states. It was noted that when plasma levels
of LS were higher than 10 ng/ml (range: 10 to 182 ng/ml), there were statistically
significant concentration-dependent increases in the percent changes of LV +dP/dt_max and
decreased percent changes of the time constant of LV relaxation (τ) and LV end-diastolic
volume (V_{ED}) in both normal and HF. The concentration responses in τ and dP/dt_max were
almost identical between normal and HF. The threshold concentration showing enhancement in $\tau$ and $dP/dt_{\text{max}}$ was around 10 ng/ml, while that in heart rate (HR) was about 30 ng/ml in normal and about 100 ng/ml in HF, indicating attenuated LS-induced HR response in HF. This efficient plasma level of LS (10 ng/ml) lasted much longer on Day 4 (LS 0.05 mg/kg) and Day 8 (LS 0.1 mg/kg) after oral LS administration (about 3.5 and 5.5 hours, respectively) when compared with Day 2 (LS 0.025 mg/kg) (about 2.5 hours).

**Dose-Related Hemodynamic and LV Functional Responses after Oral LS**

**Effect of Pacing-Induced HF.** Compared with baseline data in normal state as summarized in Tables 1 and 2 and consistent with our past reports, (Cheng, et al., 1996; Cheng, et al., 2001; Tachibana, et al., 2005) chronic ventricular rapid pacing in a canine model produced progressive LV systolic and diastolic dysfunction and LV structural remodeling. $P_{\text{ED}}$, mean LAP and $LVP_{\text{min}}$ were also significantly elevated. The $V_{\text{ES}}$ and $V_{\text{ED}}$ increased; whereas, cardiac output decreased due to a marked reduction of SV. The $W_{\text{ES}}$, $\tau$, and TSVR all significantly increased. LV contractility declined as indicated by significant reductions in $E_{\text{ES}}$ and $M_{\text{SW}}$ (Table 2 and Figures 3 and 4). The $E_{\text{ES}}/E_{\text{A}}$ ratio and LV mechanical efficiency, measured as $SW/PVA$ decreased (Table 2).

**Dose- and Time-Related Effect of Oral LS on Functional Responses.** Tables 1 and 2 show the peak effects of oral LS on hemodynamic response, plasma LS concentration, and LV functional performance in normal and HF states. During oral LS study period, in both normal and HF, no differences in baseline hemodynamics or LV function were observed. Compared with baseline data, placebo administration produced no significant changes of hemodynamics and LV function performance. In contrast, oral administration
of LS caused dose-dependent increases in peak plasma LS concentrations with correlated maximum alterations on hemodynamic and LV functional responses. As summarized in Table 1, in both normal and HF, 3 incremental dosages of oral LS produced marked arterial and venous vasodilation reflected by significant decreases in $V_{ED}$, mean LAP and TSVR. Compared with normal, in HF, LS caused significantly greater decreases in mean LAP, LV $P_{ED}$ and $LVP_{min}$. There were dose-related decreases in $V_{ES}$, both in normal and HF. The $dP/dt_{max}$ increased dose-dependently despite dose-dependent reductions of $V_{ED}$, both in normal and HF. Of note, as summarized in Table 1, compared with oral LS target doses of 0.025 mg/kg and 0.05 mg/kg, high single dose of 0.1 mg/kg caused different responses on stroke volume (SV), HR, and $P_{ES}$ in normal and HF. In HF, all 3 doses of LS significantly increased SV due to markedly reduced $V_{ES}$ with relatively unaltered HR and $P_{ES}$. In normal, similar responses on SV, HR, and $P_{ES}$ were only seen after administration of 0.025 mg/kg and 0.05mg/kg. Compared with corresponding baseline data, high-dose LS (0.1 mg/kg) failed to increase SV and significantly increased HR (+11 bpm), but decreased LV $P_{ES}$ (-7 mmHg) ($p<0.05$).

Furthermore, in both normal and HF, 3 incremental dosages of oral LS caused dose-related significantly-increased LV contractility ($E_{ES}$ and $M_{SW}$) and decreased $\tau$. As presented in Table 2 and demonstrated in Figures 1-3, these changes of $E_{ES}$ and $\tau$ paralleled the changes of plasma LS concentrations. The peak responses of $E_{ES}$ after oral LS administration were reached within 2 hours. In response to the peak effects of 3 incremental dosages of oral LS, in normals, $E_{ES}$ increased about 46%, 87%, and 125%, respectively, and, in HF, $E_{ES}$ increased about 54%, 92 %, and 142 %, respectively, with dose-dependent progressive leftward shifts of the $P_{ES}$-$V_{ES}$ relationships (Figure 3). $M_{SW}$
also increased dose-dependently both in normal and HF. In normal, $\tau$ decreased about 13%, 16%, and 21%, respectively, and, in HF, $\tau$ decreased about 12%, 18%, and 23%, respectively, indicating dose-dependent improvement in LV relaxation (Table 1). These responses were time and dose dependent. As illustrated in Figure 2, after reaching the peak values, LS-induced increase in $E_{ES}$ gradually declined, but was still apparent 5-6 hours after drug intake. Compared with $E_{ES}$, LS-induced alteration of $\tau$ was more sustained. The decrease in $\tau$ lasted up to 4.5 hours after the target doses of 0.025 and 0.05 mg/kg and up to 12 hours with the high dose of 0.1 mg/kg of LS. In both normal and HF, 3 incremental dosages of oral LS caused dose-related significant reductions in $W_{ES}$. LV-arterial coupling and $E_{ES}/E_A$ rose significantly due to increased $E_{ES}$ and decreased $E_A$, which led to significantly-improved LV mechanical efficiency ($SW/PVA$) (Table 2). Compared with normals, in HF, 3 incremental dosages of oral LS caused dose-related greater increases in $E_{ES}/E_A$, correlating with more markedly improved LV mechanical efficiency. As shown in Table 2, in response to 3 incremental dosages of oral LS, in normals, $E_{ES}/E_A$ increased about 56%, 119%, and 138%, and $SW/PVA$ rose 17%, 29%, and 33%, respectively. In HF, $E_{ES}/E_A$ increased 97%, 144%, and 194% accompanied by 37%, 47%, and 56% increases in $SW/PVA$, respectively.

**Comparison of the Effects of Oral versus IV LS and Dobutamine in Normal and HF**

To further assess the efficiency of oral LS, the peak effects of oral LS (0.05 mg/kg) were compared with intravenous LS (24 $\mu$g/kg followed by 0.2$\mu$g/kg/min) and dobutamine (2 to 10 $\mu$g/kg/min) in 6 animals before and after HF. As summarized in Table 3 and displayed in Figures 4 and 5, consistent with our past reports that these
clinically relevant dosages of IV LS and dobutamine produced positive inotropic and lusitropic actions in normals and HF. (Morimoto, et al., 2004; Tachibana, et al., 2005)

Compared with IV LS, oral LS produced similar increases in the peak plasma concentrations of LS with resultant similar increases in $E_{ES}$ and decreased the time constant of LV relaxation ($\tau$) accompanied with similar reductions of TSVR and relatively unchanged HR. These LS-induced responses were similar to the effect of dobutamine (6 $\mu$g/kg/min, IV) in normal state (Table 3 and Figure 5) except dobutamine caused a significant increase in HR. In contrast to IV and oral LS, as shown in Figure 5, after HF, the same dosage of dobutamine produced a significantly-less increase in $E_{ES}$, decrease in $\tau$ and TSVR, but still significantly increased HR, indicating blunted cardiac inotropic response and persistent chronotropic effect of dobutamine.

**Discussion**

We investigated the effects of oral LS in an animal model of HF that mimics many of the functional, structural, and neurohormonal changes of clinical HF. (Cheng, et al., 1996; Bristow, 2000; Cheng, et al., 2001; Kass and Solaro, 2006; Lenfant, 1994) We found that in both normal and HF, oral LS produced similar, dose-dependent increases in peak plasma LS concentrations paralleled with sustained dose-dependent positive inotropic and lusitropic actions accompanied by arterial and venous vasodilation. The dose of 0.1 mg/kg tended to raise plasma LS levels above 100 ng/ml and produced hypotension and tachycardia in some animals. Our results suggest that 0.025 to 0.05 mg/kg of LS would be optimal and that higher doses offer no additional benefit. These data support the
concept that oral LS might offer a new therapeutic approach for an improved HF treatment.

**Dose-Related Effect of Oral LS on Hemodynamic and LV Functional Responses**

*Positive Inotropic and Lusitropic Effects.* Despite that oral LS has been studied in several important clinical trials, the dose-effect relationship of oral LS has not been established. Previous studies of oral LS primarily reported non-invasive assessments (Hosenpud and Group, 1999; Poder, et al., 2003; Poder, et al., 2004) and right heart catheterization data (Harjola, et al., 1999). However, due to the marked simultaneous loading changes and several confounding factors such as chronic treatment with cardiovascular specific medications, lack of placebo control, and variation in severity of HF, these investigations failed to show dose-related increases in LV contractility and relaxation. The current investigation obviated the limitations of the previous studies and demonstrated a clear, positive, dose-dependent, inotropic and lusitropic effect after oral LS administration. We found that in both normal and HF, 3 incremental dosages of oral LS caused dose-related, significantly-increased LV contractility (EES and MSW) and decreased time constant of LV relaxation (τ). Importantly, these changes of EES and τ paralleled with plasma LS concentrations in proportion to the dose. The effect on LV relaxation was more sustained than could have been predicted according to plasma concentration. These results indicate that oral LS enhances myocardial contractility and relaxation to similar degrees in normal and HF that confirm and extend the findings made previously in our laboratory. (Tachibana, et al., 2005)

It was noted in a past study (Edes, et al., 1995) that a threshold concentration of LS 0.03 μM (equivalent to 8.4 ng/ml) produced a positive inotropic effect. In the present
investigation, we found that plasma levels greater than about 10 ng/ml are required to
generate clear changes in $E_{ES}$ and $\tau$, suggesting the threshold concentration for positive
inotropic and lusitropic actions of LS above 10 ng/ml. The present data, which reveal a
positive dose- and time-related improvement of LV relaxation of oral LS as opposed to
impaired relaxation by other calcium sensitizers in vitro (such as EMD 50733 and 53998,
ORG 30029, and CGP 48506), are supported by several prior experimental studies.
(Higashiyama, et al., 1995; Tachibana, et al., 2005) Enhancing the calcium sensitivity of
the contractile proteins and PDE III inhibition are believed to contribute to the LS-
induced positive inotropic action. (Edes, et al., 1995) (Haikala and Linden, 1995; Endoh,
2002) Activation of ATP-dependent potassium channels in vascular smooth muscle cells
and mitochondrial membranes (Yokoshiki, et al., 1997a; Yokoshiki, et al.,
1997b; Kaheinen, et al., 2001) as well as recent demonstrated actions of LS, such as
increased the activity of the Na$^{+}$-Ca$^{2+}$ exchanger (Hasenfuss, et al., 1998), anti-ischemic,
(Moiseyev, et al., 2002; Avgeropoulou, et al., 2005) anti-inflammatory, and anti-apoptotic
properties (Follath, et al., 2002; Parissis, et al., 2004; Trikas, et al., 2006) may also
contribute to the dose-related enhancements of LV contractile performance and relaxation.
Our findings are supported by evidence in vitro indicating that LS accelerates and does
not impair relaxation in isolated cardiac muscle from end-stage failing human hearts
(Hasenfuss, et al., 1998) and by a most recent clinical combined hemodynamic and
Doppler echocardiographic study in severe HF patients.

**LV-Arterial Coupling Effects.** Previous observations in our laboratory have
demonstrated that normal-functioning LV and arterial system are nearly optimally
coupled to produce stroke work (SW). (Little and Cheng, 1994) In the current study, we
found that during the development of HF, the $E_{ES}/E_{A}$ ratio was reduced, resulting in less than maximal SW. Single oral target doses of 0.025, 0.05 and 0.1 mg/kg of LS caused a dose-related increase in $E_{ES}$, but decreased $E_{A}$, thus causing an increase in the $E_{ES}/E_{A}$ ratio with resulting near-maximum SW after HF. $E_{A}$ rises with HR. In the current study, in both normal and HF, a single target oral dose of 0.025 and 0.05 mg/kg LS caused favorable preload and afterload reductions with unaltered HR. Increasing the dosage of LS to 0.1 mg/kg significantly increased HR in normal. In contrast, the same higher dosage of LS-induced tachycardia was significantly less in HF. Thus, the lack of chronotropic response of LS played an important role in the enhancement of the $E_{ES}/E_{A}$ ratio and LV mechanical efficiency after HF. This finding is consistent with recent clinical $E_{A}$ increases with HR. Thus, the lack of chronotropic response of LS played an important role in the enhancement of the $E_{ES}/E_{A}$ ratio and LV mechanical efficiency. This finding is consistent with recent clinical observations that LS exhibits enhanced contractility without increasing myocardial oxygen demand (Todaka, et al., 1996; Ukkonen, et al., 2000) and the induction of arrhythmias. (Niemin, et al., 2000; Follath, et al., 2002)

In this study, we found that the value of 100 ng/ml seemed to be near the upper clinical therapeutic range since higher doses (such as 0.1 mg/kg) tended to produce hypotension and increased HR. It should be noted that LS-induced positive chronotropic action can be exacerbated by several confounding factors such as inadequate filling, excessive preload reduction, and variable chronic treatment with multiple cardiovascular drugs and may worsen the long-term outcome of LS therapy in HF patients. Several moderate size trials (LIDO, RUSSLAN, and CASINO) showed mortality benefits in LS-
treated patients with decompensated HF when compared with placebo- and/or
dobutamine-treated patients. On the contrary, two recent large trials (SURVIVE and
REVIVE) failed to improve clinical outcome. As appropriately pointed out by a recent
review, (Lehtonen and Poder, 2007) the above confounding factors in SURVIVE and
REVIVE Trials may adversely alter clinical outcome by oral LS therapy in HF patients.

Effects of Oral versus Intravenous LS and Dobutamine in Normal and HF

Before HF, dobutamine (6 µg/kg/min), intravenous (IV) LS (24 µg/kg, followed
by 0.2 µg/kg/min), and oral LS (0.05 mg/kg) produced similar positive inotropic and
lusitropic action. Only dobutamine increased HR. After HF, while oral and IV LS-
induced positive inotropic and lusitropic effects persisted, the chronotropic effect was
attenuated. In contrast, dobutamine showed blunted positive inotropic and lusitropic
actions, but the positive chronotropic effect persisted. In HF, oral and IV LS caused
similar C_max levels with resultant similar favorable reductions in V_end, P_end, E_a, TSVR,
and WS_ES, correlating with similar improvement in LV-arterial coupling and LV
mechanical efficiency. This important property of LS differentiates LS from
(conventional) beta agonists. The advantages of LS over other positive inotropic agents
have been supported by several important clinical investigations in severe HF (Follath, et
al., 2002) and further encouraged by recent observations made in a Dahl/Rapp rat model
(Endoh, 2007; Louhelainen, et al., 2007) with long-term oral LS. The current findings and
past reports are consistent with the known pharmacological actions of oral LS as a
calcium sensitizer and direct vasodilator and have the potential to improve the treatment
of HF. However, studies with longer administration periods with careful dose titration,
based on individual responses, are needed for characterizing the long-term outcome effects.

**Study Limitations**

We studied an animal model with pacing-induced HF that reproduces many of the functional and neurohormonal features of clinical HF. This canine HF model demonstrated biventricular chamber dilatation with increased left and right ventricular filling pressures and striking abnormalities in systolic and diastolic function similar to those found in patients with dilated congestive cardiomyopathy. (Pagel, et al., 1997; Cheng, et al., 1996; Bristow, 2000; Lenfant, 1994) However, we cannot be certain that our results apply to HF of other causes such as hypertrophic cardiomyopathy. Additionally, to avoid the potential confounding effect of variation in severity of HF on LS-induced cardiac functional responses, studies were performed in these animals with well-established, but stable, HF (i.e., during study period, animals were not decompensated). Thus, we cannot be certain that our results directly apply to decompensated HF. However, we found that the pharmacokinetic and pharmacodynamic effects of LS in these animals were consistent with the findings of decompensated HF patients. It is important to note that experience with LS in settings other than decompensated HF is rather limited. Our observations made with stable HF are valuable and suggest the importance of pursuing further studies with oral LS for HF management, not only for severe HF, but also expanding its patient potential to include mild to moderate chronic HF.
We studied the acute effects of treatment with LS. We do not know the effect of prolonged treatment with oral LS. In humans, unlike in dogs, LS has metabolites with long elimination half-life. One of those metabolites, OR-1896, is active and has similar hemodynamic properties as LS itself and, therefore, may play an important role in the long-term effects of the drug. (Antila, et al., 2004; Lehtonen and Poder, 2007) Thus, the full potential of oral administration of LS has to be assessed in long-term studies with multiple dosing.

Conclusion

In both normal and HF, the new oral LS formulation caused dose-dependent increases in peak plasma LS concentrations parallel with dose-dependent positive inotropic and lusitropic actions accompanied with reduced vascular load due to veno- and arterial-dilation. Daily administration with single-dose 0.025 and 0.05 mg/kg LS may be optimal, both in the efficacy and safety perspective, for the treatment of normal and HF. Further investigation is warranted to clarify the clinical effectiveness of this orally-available LS capsule. The current findings support the view that oral LS is a promising approach for the improvement of HF treatment.
Acknowledgments

We gratefully acknowledge the computer programming of Dr. Ping Tan; the technical assistance of Michael Cross, Yi Zhao, Chun Xian Zhang; and the administrative support of Amanda Burnette.
REFERENCES


Edes I, Kiss E, Kitada Y, Powers FM, Papp JG, Kranias EG and Solaro RJ (1995) Effects of levosimendan, a cardiotonic agent targeted to troponin C, on cardiac function and on
phosphorylation and Ca^{2+} sensitivity of cardiac myofibrils and sarcoplasmic reticulum in guinea pig heart. *Circ Res* **77:**107-113.


Yokoshiki H, Katsube Y, Sunagawa M and Sperelakis N (1997a) Levosimendan, a novel Ca\textsuperscript{2+} sensitiser, activates the glibenclamide-sensitive K+ channel in rat arterial myocytes. 


*J Pharmacol Exp Ther* **283**:375-383.
Footnotes

This study was supported, in part, by grants from the National Institutes of Health (AA12335 to C.P.C, and HL074318 to C.P.C); and grant from the American Heart Association (0530079N to H.J.C); and Orion Pharma, Orion Corporation.
Legends for Figures

Figure 1
Efficient plasma levels and concentration-response relationship after oral levosimendan (LS) in normal and heart failure (HF) states. The percent changes of heart rate (HR), time constant of LV relaxation (τ), left ventricular (LV) end-diastolic volume (V_{ED}), and LV dp/dt_{max} were plotted to individual plasma LS concentrations detected after oral LS in normal and HF dogs. It is noted that the efficient plasma levels need to be greater than 10 ng/ml after oral LS. When plasma levels of LS were greater than 10 ng/ml, there were clear concentration-dependent increases in dP/dt_{max}, but decreased τ and V_{ED} in both normal and HF states. The efficient plasma levels that demonstrate increases in HR are about 30 ng/ml in normals and about 100 ng/ml in HF, indicating attenuated LS-induced HR responses in HF.

Figure 2
Concentration-effect relationship after incremental dosages of oral LS in normal (n=9) and HF (n=6) conscious instrumented dogs.
A) Time profile of the mean responses (mean ± SEM) of plasma LS levels and
B) LV functional performance (percent changes of contractility and relaxation) after single oral doses of LS. After 3 incremental dosages of oral LS, the plasma LS concentrations were significantly elevated within 0.5 to 2 hours in proportion to the dose. Similar peak plasma levels of LS in response to each target dosage were achieved in both normal and HF states. The changes in plasma LS concentrations paralleled the increased LV contractility (E_{ES}) and decreased in the time constant.
of LV relaxation ($\tau$). The decreased $\tau$ lasted longer (up to 4.5 hours after the target doses of 0.025 and 0.05 mg/kg and up to 12 hours after the target dose of 0.1 mg/kg of LS) than predicted based on the corresponding LS plasma concentrations.

**Figure 3**
Examples of the peak effects of incremental dosages of oral LS-induced dose-dependent increases in LV contractility before and after HF. LV $P_E-S_V$ relationships obtained from one conscious dog before and after HF during the treatment of placebo or LS administration at single oral doses of 0.025, 0.05, and 0.1 mg/kg. The $P_E-S_V$ relationship is indicated by the line. The slope and position of this line provide a load-insensitive measure of LV contractility. Compared with baselines, following placebo treatment, the LV $P_E-S_V$ relationships are relatively unchanged both before and after HF, indicating no change in LV contractility. In contrast, after incremental dosages of LS administration, there are progressive leftward shifts of the $P_E-S_V$ relationship with increased slope, indicating that oral LS produces dose-dependent increases in LV contractility both before and after HF.

**Figure 4**
Comparison of the peak effects of oral (0.05 mg/kg) and intravenous LS (24 $\mu$g/kg followed by 0.2 $\mu$g/kg/min) administration in normal and HF states.

A) Mean (±SEM) of the peak plasma levels of LS after oral (n=6) or intravenous LS (n=3 for normal and n=6 for HF). Oral and intravenous LS administration
produced similar increases in the peak plasma LS concentrations in dogs before and after HF.

B) \( P_{ES} - V_{ES} \) relationships obtained from two different conscious dogs, one normal (a) and one HF (b) after LS. Following oral or intravenous LS administration, there were similar leftward shifts of the \( P_{ES} - V_{ES} \) relationship with increased slope, indicating similar increases in LV contractility in both normal and HF states.

**Figure 5**

Examples of comparison of the peak effects of oral LS (0.05 mg/kg) and dobutamine (DOB) (6 \( \mu \)g/kg/min) on LV \( P_{ES} - V_{ES} \) relationship in normal and HF states. LV \( P_{ES} - V_{ES} \) relationships obtained from the same conscious dog before and after HF with oral LS and DOB treatments. Before HF, oral LS produced marked increases in LV contractility measured by the slopes of \( P_{ES} - V_{ES} \) relationship (\( E_{ES} \)), which were similar to DOB (6 \( \mu \)g/kg/min, IV). After HF, the cardiac response to DOB was blunted; however, LS positive inotropy persisted.
<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Placebo</th>
<th>Day 2</th>
<th>LS1 (0.025mg/kg)</th>
<th>Baseline</th>
<th>Day 4</th>
<th>LS2 (0.05mg/kg)</th>
<th>Baseline</th>
<th>Day 8</th>
<th>LS3 (0.1mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hemodynamics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>114 ± 11</td>
<td>117 ± 11</td>
<td>116 ± 11</td>
<td>118 ± 13</td>
<td>115 ± 13</td>
<td>119 ± 11‡</td>
<td>115 ± 11</td>
<td>126 ± 12‡†‡§</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak +dP/dt, mm Hg/s</td>
<td>2238 ± 377</td>
<td>2187 ± 339</td>
<td>2217 ± 369</td>
<td>2593 ± 451†</td>
<td>2226 ± 366</td>
<td>2822 ± 563‡</td>
<td>2213 ± 376</td>
<td>2892 ± 638†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak -dP/dt, mm Hg/s</td>
<td>-2065 ± 237</td>
<td>-2012 ± 240</td>
<td>-2020 ± 231</td>
<td>-2183 ± 226‡</td>
<td>-2062 ± 226</td>
<td>-2237 ± 242‡</td>
<td>-2049 ± 187</td>
<td>-2195 ± 340†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LV end-diastolic pressure, mm Hg</td>
<td>10.5 ± 1.9</td>
<td>9.9 ± 2.2</td>
<td>10.6 ± 1.7</td>
<td>7.7 ± 1.6†</td>
<td>9.9 ± 1.5</td>
<td>7.4 ± 2.1‡‡</td>
<td>10.2 ± 1.7</td>
<td>5.6 ± 2.8†‡</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LV end-systolic pressure, mm Hg</td>
<td>104 ± 5.4</td>
<td>101 ± 6.6</td>
<td>102 ± 7.4</td>
<td>103 ± 7.7</td>
<td>104 ± 7.8</td>
<td>101 ± 7.5‡</td>
<td>104 ± 4.7</td>
<td>97 ± 4.7*†‡</td>
<td></td>
<td></td>
</tr>
<tr>
<td>minimum LVP, mm Hg</td>
<td>2.0 ± 1.1</td>
<td>2.1 ± 1.5</td>
<td>1.7 ± 1.5</td>
<td>-0.2 ± 2.2*‡</td>
<td>1.6 ± 1.2</td>
<td>-0.5 ± 2.8*‖§</td>
<td>1.7 ± 0.9</td>
<td>-2.0 ± 2.1*‖§</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean LAP, mm Hg</td>
<td>5.8 ± 1.0</td>
<td>5.6 ± 1.4</td>
<td>6.3 ± 1.1</td>
<td>4.0 ± 1.9*</td>
<td>5.9 ± 1.2</td>
<td>3.5 ± 1.6*†</td>
<td>5.3 ± 1.4</td>
<td>2.7 ± 1.0**†§</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LV end-diastolic volume, mL</td>
<td>42.2 ± 11.0</td>
<td>41.5 ± 11.8</td>
<td>41.9 ± 11.3</td>
<td>39.9 ± 12.0*†</td>
<td>42.1 ± 11.5</td>
<td>38.3 ± 10.1*†</td>
<td>42.0 ± 11.8</td>
<td>35.8 ± 9.5*‖§</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LV end-systolic volume, mL</td>
<td>30.3 ± 9.8</td>
<td>30.1 ± 10.2</td>
<td>30.1 ± 10.2</td>
<td>27.6 ± 10.2*†</td>
<td>30.2 ± 10.3</td>
<td>25.6 ± 8.1*†‡</td>
<td>30.1 ± 10.5</td>
<td>24.2 ± 9.5*‖§</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stroke volume, mL</td>
<td>11.7 ± 3.0</td>
<td>11.3 ± 3.2</td>
<td>11.8 ± 3.4</td>
<td>12.5 ± 3.5*†</td>
<td>11.9 ± 2.7</td>
<td>13.3 ± 3.3*†</td>
<td>11.9 ± 3.5</td>
<td>11.8 ± 4.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TSVR, mmHg/mL/min</td>
<td>0.083 ± 0.021</td>
<td>0.081 ± 0.021</td>
<td>0.084 ± 0.028</td>
<td>0.077 ± 0.026*</td>
<td>0.081 ± 0.023</td>
<td>0.070 ± 0.023*†</td>
<td>0.086 ± 0.025</td>
<td>0.072 ± 0.020*‖§</td>
<td></td>
<td></td>
</tr>
<tr>
<td>τ, ms</td>
<td>29.2 ± 1.5</td>
<td>28.8 ± 1.6</td>
<td>29.5 ± 1.7</td>
<td>25.6 ± 1.9*†</td>
<td>29.3 ± 1.5</td>
<td>24.7 ± 2.5*‖§</td>
<td>29.7 ± 1.8</td>
<td>23.5 ± 1.6*‖§</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WSES, g/cm²</td>
<td>60.5 ± 15.6</td>
<td>59.0 ± 17.3</td>
<td>59.7 ± 18.6</td>
<td>55.9 ± 18.3*†</td>
<td>60.6 ± 18.9</td>
<td>52.1 ± 16.5*‖§</td>
<td>60.3 ± 16.7</td>
<td>47.0 ± 13.5*‖§</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Plasma LS Levels</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt;</td>
<td>-</td>
<td>-</td>
<td>&lt;</td>
<td>34.6 ± 3.6</td>
<td>&lt;</td>
<td>66.8 ± 9.5‡</td>
<td>&lt;</td>
<td>123.2 ± 12.7‡§</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T&lt;sub&gt;max&lt;/sub&gt;</td>
<td>-</td>
<td>-</td>
<td>&lt;</td>
<td>2.2±0.6</td>
<td>&lt;</td>
<td>1.2±0.3‡</td>
<td>&lt;</td>
<td>2.1 ± 0.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

LS, Levosimendan; LV, left ventricular; LAP, left atrial pressure; TSVR, total systemic vascular resistance; τ, time constant of LV relaxation; WSES, mean end-systolic circumference stress of LV. C<sub>max</sub>, peak plasma concentration of LS; T<sub>max</sub>, time to reach C<sub>max</sub>. No blood collections were done for plasma LS measurements; <, values of LS concentration below the lower limit of quantitation LLOQ (0.200 ng/ml). n= 9, number of dogs. Hemodynamic values are mean ± SD. LS plasma concentration values and T<sub>max</sub> are mean ± SEM. * p<0.05, LS vs. corresponding baseline; † p<0.05, LS vs. placebo; ‡ p<0.05, LS2 vs. LS1, and LS3 vs. LS1; § LS3 vs. LS2.
### Table 1B. Peak Effects of Three Incremental Dosages of Oral LS on Steady-State Hemodynamics and Plasma LS Concentrations in HF State

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 4</th>
<th>Day 8</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Placebo</td>
<td>LS1 (0.025mg/kg)</td>
<td>Baseline</td>
</tr>
<tr>
<td><strong>Hemodynamics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>127 ± 15</td>
<td>124 ± 15</td>
<td>126 ± 11</td>
<td>121 ± 13</td>
</tr>
<tr>
<td>Peak +dP/dt, mm Hg/s</td>
<td>1431 ± 333</td>
<td>1409 ± 307</td>
<td>1436 ± 303</td>
<td>1613 ± 390†</td>
</tr>
<tr>
<td>LV end-diastolic pressure, mm Hg</td>
<td>27.3 ± 4.2</td>
<td>26.7 ± 3.7</td>
<td>27.2 ± 4.2</td>
<td>23.5 ± 3.9†</td>
</tr>
<tr>
<td>LV end-systolic pressure, mm Hg</td>
<td>97 ± 7.8</td>
<td>96 ± 8.4</td>
<td>98 ± 7.7</td>
<td>97 ± 9.2</td>
</tr>
<tr>
<td>Mean LAP, mm Hg</td>
<td>22.4 ± 3.9</td>
<td>22.4 ± 3.8</td>
<td>22.0 ± 4.0</td>
<td>18.5 ± 5.0*</td>
</tr>
<tr>
<td>LV end-diastolic volume, mL</td>
<td>51.4 ± 15.5</td>
<td>51.2 ± 15.5</td>
<td>51.0 ± 15.2</td>
<td>49.1 ± 15.6†</td>
</tr>
<tr>
<td>LV end-systolic volume, mL</td>
<td>42.8 ± 13.7</td>
<td>42.4 ± 13.3</td>
<td>42.4 ± 13.4</td>
<td>38.6 ± 12.9†</td>
</tr>
<tr>
<td>Stroke volume, mL</td>
<td>8.6 ± 2.1</td>
<td>8.8 ± 2.5</td>
<td>8.6 ± 2.3</td>
<td>10.5 ± 3.3†</td>
</tr>
<tr>
<td>TSVR, mmHg/mL/min</td>
<td>0.094 ± 0.019</td>
<td>0.095 ± 0.020</td>
<td>0.098 ± 0.022</td>
<td>0.084 ± 0.022†</td>
</tr>
<tr>
<td>τ, ms</td>
<td>44.5 ± 6.8</td>
<td>44.7 ± 7.2</td>
<td>44.0 ± 6.9</td>
<td>38.9 ± 6.8†</td>
</tr>
<tr>
<td>WSES, g/cm²</td>
<td>73.6± 19.5</td>
<td>73.3± 19.1</td>
<td>74.8± 21.2</td>
<td>69.3± 20.5*</td>
</tr>
</tbody>
</table>

| **Plasma LS Levels** |       |       |       |       |       |       |
| C<sub>max</sub> | - | - | < | 38.3 ± 4.4 | < | 71.5 ± 13.7† | < | 137.4 ± 15.9§ |
| T<sub>max</sub> | - | - | < | 2.3±0.8 | < | 1.8±0.6 | < | 1.5±0.3 |

LS, Levosimendan; LV, left ventricular; LAP, left atrial pressure; TSVR, total systemic vascular resistance; τ, time constant of LV relaxation; WSES, mean end-systolic circumference stress of LV. C<sub>max</sub>, peak plasma concentration of LS; T<sub>max</sub>, time to reach C<sub>max</sub>. No blood collections were done for plasma LS measurements; <, values of concentration below the lower limit of quantitation LLOQ (0.200 ng/ml). n= 7, numbers of dogs. Hemodynamic values are mean ± SD; LS plasma concentration values and T<sub>max</sub> are mean ± SEM. * p<0.05, LS vs. corresponding baseline; † p<0.05, LS vs. placebo; ‡ p<0.05, LS2 vs. LS1, and LS3 vs. LS1; § LS3 vs. LS2.
# TABLE 2. PEAK EFFECTS OF INCREMENTAL DOSAGES OF ORAL LS ON LV P-V RELATIONS, LV-ARTERIAL COUPLING, AND MECHANICAL EFFICIENCY IN NORMAL AND HF

<table>
<thead>
<tr>
<th></th>
<th>Normal (n=9)</th>
<th></th>
<th></th>
<th>HF (n=7)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 2</td>
<td>Day 4</td>
<td>Day 8</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Baseline</td>
<td>Placebo</td>
<td>Baseline</td>
<td>LS1</td>
<td>LS2</td>
<td>LS3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Baseline (0.025mg/kg)</td>
<td></td>
<td>Baseline (0.05mg/kg)</td>
<td>Baseline (0.1mg/kg)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Baseline</td>
<td>LS2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Baseline (0.05mg/kg)</td>
<td></td>
<td>Baseline</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Baseline</td>
<td>LS3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Baseline (0.1mg/kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>EES, mm Hg/ml</strong></td>
<td>5.6 ± 1.3</td>
<td>5.7 ± 0.2</td>
<td>5.6 ± 1.3</td>
<td>8.2 ± 1.9†</td>
<td>5.6 ± 1.3</td>
<td>10.5 ± 1.9†</td>
</tr>
<tr>
<td><strong>Msvx, mm Hg</strong></td>
<td>69.9 ± 7.4</td>
<td>68.2 ± 7.9</td>
<td>68.9 ± 9.4</td>
<td>86.3 ± 4.9†</td>
<td>67.0 ± 6.9</td>
<td>90.8 ± 12.0†</td>
</tr>
<tr>
<td><strong>Ees / Ea</strong></td>
<td>0.61 ± 0.13</td>
<td>0.62 ± 0.16</td>
<td>0.63 ± 0.18</td>
<td>0.98 ± 0.25†</td>
<td>0.62 ± 0.11</td>
<td>1.36 ± 0.32†</td>
</tr>
<tr>
<td><strong>Sw / Pva</strong></td>
<td>0.58 ± 0.05</td>
<td>0.58 ± 0.06</td>
<td>0.58 ± 0.07</td>
<td>0.68 ± 0.06†</td>
<td>0.58 ± 0.04</td>
<td>0.75 ± 0.05†</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Ees, mm Hg/ml</strong></td>
<td>3.6 ± 0.8</td>
<td>3.7 ± 0.9</td>
<td>3.7 ± 0.8</td>
<td>5.7 ± 1.1†</td>
<td>3.7 ± 0.7</td>
<td>7.1 ± 1.6†</td>
</tr>
<tr>
<td><strong>Msvx, mm Hg</strong></td>
<td>42.7 ± 3.7</td>
<td>43.8 ± 6.5</td>
<td>44.9 ± 3.7</td>
<td>58.5 ± 4.9†</td>
<td>44.3 ± 5.1</td>
<td>65.1 ± 5.2†</td>
</tr>
<tr>
<td><strong>Ees / Ea</strong></td>
<td>0.32 ± 0.07</td>
<td>0.33 ± 0.08</td>
<td>0.31 ± 0.06</td>
<td>0.61 ± 0.14†</td>
<td>0.32 ± 0.07</td>
<td>0.78 ± 0.27†</td>
</tr>
<tr>
<td><strong>Sw / Pva</strong></td>
<td>0.44 ± 0.07</td>
<td>0.44 ± 0.06</td>
<td>0.43 ± 0.06</td>
<td>0.59 ± 0.05†</td>
<td>0.43 ± 0.06</td>
<td>0.63 ± 0.08†</td>
</tr>
</tbody>
</table>

LS, Levosimendan; EES, slope of linear PES-VES relation; Msvx, stroke work-VES relation; Ea, arterial elastance; SW, stroke work; PVA, pressure-volume area. n, number of dogs. Values are mean ± SD. * p<0.05, LS vs. corresponding baseline; † p<0.05, LS vs. placebo; ‡ p<0.05, LS2 vs. LS1 and LS3 vs. LS1; § LS3 vs. LS2.
Table 3. Comparison of Oral LS, Intravenous LS and Dobutamine on the Peak Hemodynamic Responses before and after HF

<table>
<thead>
<tr>
<th></th>
<th>Oral LS (0.05mg/kg)</th>
<th>Intravenous LS (24 µg/kg, plus 0.2 µg/kg/min)</th>
<th>Dobutamine (DOB) (6-8 µg/kg/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>After LS</td>
<td>Baseline</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>119 ± 14</td>
<td>120 ± 11</td>
<td>114 ± 8</td>
</tr>
<tr>
<td>EES (mmHg/ml)</td>
<td>5.4 ± 1.5</td>
<td>10.1 ± 2.4*</td>
<td>5.5 ± 1.4</td>
</tr>
<tr>
<td>Time constant of relaxation (ms)</td>
<td>28.6 ± 1.2</td>
<td>23.5 ± 2.0*</td>
<td>29.8 ± 1.3</td>
</tr>
<tr>
<td>TSVR (mmHg/ml/min)</td>
<td>0.074 ± 0.02</td>
<td>0.063 ± 0.02*</td>
<td>0.071 ± 0.02</td>
</tr>
</tbody>
</table>

Before HF
(n=6)

After HF
(n=6)

| Heart rate (beats/min) | 133 ± 19† | 138 ± 15 | 124 ± 13† | 124 ± 19 | 122 ± 17† | 128 ± 13* |
| EES (mmHg/ml)          | 3.6 ± 0.7† | 7.2 ± 1.5* | 3.6 ± 0.8† | 7.0 ± 1.4* | 3.9 ± 0.9† | 4.8 ± 1.5‡‡§ |
| Time constant of relaxation (ms) | 43.2 ± 5.4† | 34.6 ± 4.5* | 45.2 ± 8.4 | 35.7 ± 8.0* | 45.3 ± 1.8† | 41.5 ± 2.9‡‡§ |
| TSVR (mmHg/ml/min)     | 0.091 ± 0.02† | 0.072 ± 0.02* | 0.088 ± 0.02† | 0.071 ± 0.02* | 0.092 ± 0.01† | 0.084 ± 0.01*‡‡§ |

LS, Levosimendan; EES, slope of linear PES-VES relation. TSVR, total systemic vascular resistance. n, numbers of dogs. Hemodynamic values are mean ± SD; * p<0.05, after drug vs. corresponding baseline; † p<0.05, HF baseline vs. normal baseline; ‡ p<0.05, HF DOB vs. normal DOB; § HF DOB vs. HF po LS.
Figure 1
A. Mean (± SEM) Response of Plasma LS Concentrations

B. Mean (± SEM) Response of LV Contractility and Relaxation

Figure 2
A. Mean (±SEM) of the Peak Plasma Levels of LS

![Bar graphs showing plasma levels of LS in Normal and Heart Failure conditions.]

B. Examples of $P_{ES} - V_{ES}$ Relation Responses

![Graphs showing LV pressure vs LV volume for Oral LS and Intravenous LS in Normal and Heart Failure (HF) conditions.]

Figure 4