Orally Available Levosimendan Dose-Related Positive Inotropic and Lusitropic Effect in Conscious Chronically Instrumented Normal and Heart Failure Dogs

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ABSTRACT

Levosimendan (LS), a Ca^{2+} sensitizer, is presently limited to i.v. 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 (day 2), 0.05 (day 4), and 0.1 (day 8) 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 three incremental dosages of oral LS, the peak plasma LS concentrations (34.6, 66.8, and 123.2 ng/ml in normal and 38.3, 71.5, and 137.4 ng/ml in HF) were achieved within 2 h in proportion to the dose, parallel to an increased LV contractility (normal, from 5.7 mm Hg/ml placebo to 8.2, 10.5, and 12.6 mm Hg/ml; HF, from 3.7 mm Hg/ml placebo to 5.7, 7.1, and 8.7 mm Hg/ml), and decreased time constant of LV relaxation (τ) (normal, from 28.8 ms of placebo to 25.6, 24.7, and 23.5 mm Hg/ml; HF, from 44.7 ms of placebo to 38.9, 36.4, and 34.6 ms). 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 LS in normal dogs. In conclusion, oral LS produces vasodilatation and dose-dependent augmentation in LV contractility and relaxation both in normal and HF.

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 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 down-regulation 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+} (Harkin et al., 1995; Pagel et al., 1997); whereas at higher concentrations, its action as a phosphodiesterase 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 left ventricular (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 (En-doh, 2007; Lehtonen and Poder, 2007). In contrast to dobutamine, LS does not increase myocardial oxygen demand and has anti-ischemic and antiarrhythmic actions (Pagel et al., 1997; Ukkonen et al., 2000; Moiseyev et al., 2002; Avgere-poulou et al., 2005). In patients with severe HF, LS exerts anti-inflammatory and antiapoptotic properties (Follath et al., 2002; Parissis et al., 2004; Trikas et al., 2006). 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 i.v. 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 noninvasive assessment (Hosen-pud and Group, 1999; Pöder et al., 2003, 2004) 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.

Materials and Methods

Instrumentation

This investigation was approved by the Institutional Animal Care and Use Committee and conforms to the Guide for the Care and Use Laboratory Animals (Institute of Laboratory Animal Resources, 1996). Nine healthy, adult, heartworm-negative mongrel dogs (body weight, 24–35 kg) were chronically instrumented to measure three LV internal dimensions, LV pressure (LVP), and left atrial pressure (LAP). Myocardial leads (model 4312; Cardiac Pacemakers, Minneapolis, MN) were implanted within the myocardium of the right ventricle and right atrium, and the leads were attached to unipolar multiprogrammable pacemakers (model 8329; Medtronic, Minneapolis, MN) positioned under the skin in the chest. Hydraulic occluders were placed around the venae cavae by a technique described previously (Cheng et al., 1996; Morimoto et al., 2004; Tachibana et al., 2005).

Effect of Oral LS.

Following an 8-day study period, animals received three 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. Variables loaded LV-P-V loops were generated by sudden, transient occlusion of the vena cava (VCO) as described previously (Tachibana et al., 2005). Then, a placebo capsule was given to the animals, and food intake was generally allowed after 1 to 2 h after oral drug intake. Hemodynamic measurements were repeated at 0.5, 1, 1.5, 2, 3, 4, 6, 8, and 12 h 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 LS was given to the animals. Hemody-namic measurements and blood sample collections were repeated at 0.5, 1, 1.5, 2, 3, 4, 6, 8, and 12 h 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 (day 4) and 0.1 (day 8) mg/kg LS was given to the animals, data were collected, and blood sampling was performed as described in day 2.

Effect of i.v. LS.

After baseline hemodynamic data and blood sample collections, animals received i.v. 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 i.v. Dobutamine.

After baseline hemodynamic data collection, animals received dobutamine i.v. infusion (2, 4, 6, 8, and 10 μg/kg/min at 10-min intervals). Data were again obtained.

Induction of Stable HF

After the completion of normal studies, rapid right ventricular pacing was initiated. As described previously (Cheng et al., 2001), the pacing protocol producing a stable degree of HF was performed with rapid right ventricular pacing [210–240 beats per minute (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 nonpacing period had increased by more than 15 mm Hg over the prepacing data and control blood samples for oral and i.v. LS were collected.

Studies after HF

In all of the animals, LS studies started after the onset of HF plus 1 week of pacing at 190 bpm (i.e., during the stable HF period). In brief, 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 1 h. The HF baseline hemodynamic data and control blood samples for oral and i.v. LS were collected.
Then, oral LS (from days 1–8), i.v. 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 i.v. 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 described previously (Cheng et al., 1996, 2001; 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), LV end-systolic V (VES), 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-s recording period spanning multiple respiratory cycles. The τ value was determined by analyzing the exponential time constant of the isovolumic fall of LV P by the Weiss equation. The rate of LV relaxation was obtained from computer-assisted graphical analysis of dP/dtmax and decreased percentage of changes of the time constant of LV relaxation (τ) and VED in both normal and heart failure (HF) animals. The concentration-response relationship after oral LS in normal and HF animals, after three incremental dosages of oral LS, the plasma LS concentrations were significantly elevated above 10 to 30 ng/ml within 0.5 to 2 h. Similar peak plasma concentrations of LS (Cmax) were reached within 2 h (Tmax) in proportion to the dose (Table 1). The similar pharmacokinetic parameters (such as Cmax and Tmax) of LS after incremental dosages of oral LS administration showed that no accumulation of LS occurred with any target dosing interval.

Data were summarized as mean ± S.D. (or indicated as mean ± S.E.M.). 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 analysis of variance (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 analysis of covariance on the outcome measures adjusted for baseline values. Repeated measured ANOVA was used for the time effect with separate paired Student’s 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 nine 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 nine normal and seven 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 six animals that had been collected with the three drug treatments (oral LS, i.v. 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 days 2, 4, and 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 Figs. 1 and 2A, in both normal and HF animals, after three incremental dosages of oral LS, the plasma LS concentrations were significantly elevated above 10 to 30 ng/ml within 0.5 to 2 h. Similar peak plasma concentrations of LS (Cmax) were reached within 2 h (Tmax) in proportion to the dose (Table 1). The similar pharmacokinetic parameters (such as Cmax and Tmax) 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, 10–182 ng/ml), there were statistically significant concentration-dependent increases in the percentage of changes of LV +dP/dtmax and decreased percentage of changes of the time constant of LV relaxation (τ) and VED in both normal and HF. The concentration responses in τ and dP/dtmax were almost identical between normal and HF. The threshold concentration showing enhancement in τ and dP/dtmax was approximately 10 ng/ml, whereas that in heart rate (HR) was approximately 30 ng/ml in normal and approximately 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 days 4 (LS 0.05 mg/kg) and 8 (LS 0.1 mg/kg) after oral LS administration (approximately 3.5 and 5.5 h, respectively) compared with day 2 (LS 0.025 mg/kg) (approximately 2.5 h).

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, 2001; Tachibana et al., 2005), chronic ventricular rapid pacing in a canine model produced progressive LV systolic and diastolic dysfunction and LV structural remodel-
TABLE 1
Peak effects of incremental dosages of oral LS on steady-state hemodynamics and plasma LS concentrations in normal and HF states

<table>
<thead>
<tr>
<th></th>
<th>Normal state</th>
<th>HF state</th>
<th>Plasma LS levels</th>
<th>Oral Levosimendan Improves Cardiac Performance in HF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Placebo</td>
<td>Baseline LS1 (0.025 mg/kg)</td>
<td>Baseline LS2 (0.05 mg/kg)</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>114 ± 11</td>
<td>117 ± 11</td>
<td>116 ± 11</td>
<td>118 ± 13</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>
</tr>
<tr>
<td>Peak – dP/dt (mm Hg/s)</td>
<td>-2065 ± 237</td>
<td>-2025 ± 240</td>
<td>-2020 ± 231</td>
<td>-2183 ± 226†+++</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>
</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>
</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>
</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>
</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>
</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>
</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>
</tr>
<tr>
<td>TSVR (mm Hg/ml/min)</td>
<td>0.083 ± 0.021</td>
<td>0.081 ± 0.021</td>
<td>0.084 ± 0.028</td>
<td>0.070 ± 0.026*†‡§</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>
</tr>
<tr>
<td>WSLS (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>
</tr>
<tr>
<td>Plasma LS levels</td>
<td>Cmax (ng/ml)</td>
<td>–</td>
<td>&lt; 34.6 ± 3.6</td>
<td>&lt; 66.8 ± 9.5†</td>
</tr>
<tr>
<td>Tmax (h)</td>
<td>–</td>
<td>&lt; 2.2 ± 0.6</td>
<td>&lt; &lt;!--snip--&gt;/H11006</td>
<td>&lt; 66.8 ± 9.5†</td>
</tr>
</tbody>
</table>

† time constant of LV relaxation; Cmax peak plasma concentration of LS; Tmax time to reach Cmax; – no blood collections were done for plasma LS measurements; <, values of LS concentration below the lower limit of quantitation (0.200 ng/ml); n = 9, number of dogs, normal state; n = 7, HF state. Hemodynamic values are mean ± S.D. LS plasma concentration values and Tmax are mean ± S.E.M.

* p < 0.05, LS vs. corresponding baseline.
† p < 0.05, LS vs. placebo.
‡ p < 0.05, LS2 vs. LS1 and LS3 vs. LS1.
§ LS1 vs. LS2.
eling. PED, mean LAP, and LVPmin were also significantly elevated. The VES and VED increased, whereas cardiac output decreased due to a marked reduction of SV. The WSES, MSW, and TSVR all significantly increased. LV contractility declined as indicated by significant reductions in EES and MSW (Table 2; Figs. 3 and 4). The EES/EA 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 the 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 functional 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, compared with oral LS target doses of 0.025 and 0.05 mg/kg, high single dose of 0.1 mg/kg caused different responses on SV, HR, and PES in normal and HF in normal and HF. LS caused significantly greater decreases in mean LAP, LV PED, and LVPmin. Compared with normal, in HF, LS caused significantly greater decreases in mean LAP, LV PED, and LVPmin. There were dose-related decreases in VES, both in normal and HF. The dP/dtmax increased dose-dependently despite dose-dependent reductions of VED, both in normal and HF. It is noteworthy that as summarized in Table 1, compared with oral LS target doses of 0.025 and 0.05 mg/kg, high single dose of 0.1 mg/kg caused different responses on SV, HR, and PES in normal and HF. LS caused significantly greater decreases in mean LAP, LV PED, and LVPmin. In HF, all three doses of LS significantly increased SV due to markedly reduced VED with relatively unaltered HR and PED. In normal, similar responses on SV, HR, and PES were only seen after administration of 0.025 and 0.05 mg/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 PES (-7 mm Hg) (p < 0.05).

Furthermore, in both normal and HF, three incremental dosages of oral LS caused dose-related significantly increased LV contractility (EES and MSW) and decreased τ. As presented in Table 2 and demonstrated in Figs. 1 to 3, these changes of EES and τ paralleled the changes of plasma LS concentrations. The peak responses of EES after oral LS administration were reached within 2 h. In response to the peak effects of three incremental dosages of oral LS, in normals, EES increased approximately 46, 87, and 125%, respectively, and, in HF, EES increased approximately 54, 92, and 142%, respectively, with dose-dependent progressive leftward shifts of the PES-VES relationships (Fig. 3). MSW also increased dose-dependently both in normal and HF. In normal, τ decreased approximately 13, 16, and 21%, respectively, and, in HF, τ decreased approximately 12, 18, and 23%, respectively, indicating dose-dependent improvement.
Fig. 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 ± S.E.M.) of plasma LS levels. B, LV functional performance (percentage of changes of contractility and relaxation) after single oral doses of LS. After three incremental dosages of oral LS, the plasma LS concentrations were significantly elevated within 0.5 to 2 h 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 (Ees) and decreased in the time constant of LV relaxation (τ). The decreased τ lasted longer (up to 4.5 h after the target doses of 0.025 and 0.05 mg/kg and up to 12 h after the target dose of 0.1 mg/kg LS) than predicted based on the corresponding LS plasma concentrations.
in LV relaxation (Table 1). These responses were time- and dose-dependent. As illustrated in Fig. 2, after reaching the peak values, LS-induced increase in EES gradually declined but was still apparent 5 to 6 h after drug intake. Compared with EES, LS-induced alteration of \( \Delta H9270 \) was more sustained. The decrease in \( \Delta H9270 \) lasted up to 4.5 h after the target doses of 0.025 and 0.05 mg/kg and up to 12 h with the high dose of 0.1 mg/kg LS. In both normal and HF, three incremental dosages of oral LS caused dose-related significant reductions in WS\(_{EES}\) LV-arterial coupling and E\(_{EES}/E_A\) rose significantly due to increased E\(_{EES}\) and decreased E\(_A\), which led to significantly improved LV mechanical efficiency (SW/PVA) (Table 2). Com-

### Table 2

<table>
<thead>
<tr>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 4</th>
<th>Day 8</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td><strong>Placebo</strong></td>
<td><strong>Baseline (0.025 mg/kg)</strong></td>
<td><strong>Baseline (0.05 mg/kg)</strong></td>
</tr>
<tr>
<td>Normal ((n = 9))</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E(_{EES}) (mm Hg/ml)</td>
<td>5.6 ± 1.3</td>
<td>5.7 ± 0.2</td>
<td>8.2 ± 1.9</td>
</tr>
<tr>
<td>M(_{EES}) (mm Hg)</td>
<td>69.9 ± 7.4</td>
<td>68.2 ± 7.9</td>
<td>68.9 ± 9.4</td>
</tr>
<tr>
<td>E(_{EES}/E_A)</td>
<td>0.61 ± 0.13</td>
<td>0.62 ± 0.16</td>
<td>0.63 ± 0.18</td>
</tr>
<tr>
<td>SW/PVA</td>
<td>0.58 ± 0.05</td>
<td>0.58 ± 0.06</td>
<td>0.58 ± 0.07</td>
</tr>
<tr>
<td>HF ((n = 7))</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E(_{EES}) (mm Hg/ml)</td>
<td>3.6 ± 0.8</td>
<td>3.7 ± 0.9</td>
<td>3.7 ± 0.8</td>
</tr>
<tr>
<td>M(_{EES}) (mm Hg)</td>
<td>42.7 ± 3.7</td>
<td>43.8 ± 6.5</td>
<td>44.9 ± 3.7</td>
</tr>
<tr>
<td>E(_{EES}/E_A)</td>
<td>0.32 ± 0.07</td>
<td>0.33 ± 0.08</td>
<td>0.31 ± 0.06</td>
</tr>
<tr>
<td>SW/PVA</td>
<td>0.44 ± 0.07</td>
<td>0.44 ± 0.06</td>
<td>0.43 ± 0.06</td>
</tr>
</tbody>
</table>

\( n \), number of dogs. Values are mean ± S.D.

\( * \) \( p < 0.05, \) LS vs. corresponding baseline.

\( \dagger \) \( p < 0.05, \) LS vs. placebo.

\( \ddagger \) \( p < 0.05, \) LS2 vs. LS1 and LS3 vs. LS1.

\( \S \) LS3 vs. LS2.

**Fig. 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\(_{EES}\)-V\(_{EES}\) 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\(_{EES}\)-V\(_{EES}\) relationship is indicated by the line. The slope and position of this line provide a load-insensitive measure of LV contractility. Compared with baselines, after placebo treatment, the LV P\(_{EES}\)-V\(_{EES}\) 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\(_{EES}\)-V\(_{EES}\) relationship with increased slope, indicating that oral LS produces dose-dependent increases in LV contractility both before and after HF.
pared with normals, in HF, three 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 three incremental dosages of oral LS, in normals, $E_{ES}/E_A$ increased approximately 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.

**Fig. 4.** Comparison of the peak effects of oral (0.05 mg/kg) and i.v. (24 μg/kg followed by 0.2 μg/kg/min) LS administration in normal and HF states. A, mean (±S.E.M.) of the peak plasma levels of LS after oral ($n = 6$) or i.v. ($n = 3$ for normal and $n = 6$ for HF) LS. Oral and i.v. 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 and one HF after LS. After oral or i.v. 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.
Comparison of the Effects of Oral versus i.v. 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 i.v. LS (24 μg/kg followed by 0.2 μg/kg/min) and dobutamine (2–10 μg/kg/min) in six animals before and after HF, as summarized in Table 3 and displayed in Figs. 4 and 5, consistent with our past reports that these clinically relevant dosages of i.v. LS and dobutamine produced positive inotropic and lusitropic actions in normals and HF (Morimoto et al., 2004; Tachibana et al., 2005). Compared with i.v. LS, oral LS produced similar increases in the peak plasma concentrations of LS with resultant similar increases in $E_{\text{ES}}$ 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 μg/kg/min i.v.) in normal state (Table 3; Fig. 5), except dobutamine caused a significant increase in HR. In contrast to i.v. and oral LS, as shown in Fig. 5, after HF, the same dosage of dobutamine produced a significantly less increase in $E_{\text{ES}}$, 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 (Lenfant, 1994; Cheng et al., 1996; Bristow, 2000; Cheng et al., 2001; Kass and Solaro, 2006). We found that in both normal and HF, oral LS produced similar, dose-dependent increases in peak plasma concentrations of LS 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 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.

### Table 3

Comparison of oral LS, i.v. LS, and dobutamine on the peak hemodynamic responses before and after HF

<table>
<thead>
<tr>
<th></th>
<th>Oral LS (0.05 mg/kg)</th>
<th>Intravenous LS (24 mg/kg, plus 0.2 mg/kg/min)</th>
<th>DOB (6–8 mg/kg/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>After LS</td>
<td>Baseline</td>
</tr>
<tr>
<td>Before HF ($n = 6$)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>$119 \pm 14$</td>
<td>$120 \pm 11$</td>
<td>$114 \pm 8$</td>
</tr>
<tr>
<td>$E_{\text{ES}}$ (mm Hg/ml)</td>
<td>$5.4 \pm 1.5$</td>
<td>$10.1 \pm 2.4^*$</td>
<td>$5.5 \pm 1.4$</td>
</tr>
<tr>
<td>Time constant of relaxation (ms)</td>
<td>$28.6 \pm 1.2$</td>
<td>$25.3 \pm 2.0^*$</td>
<td>$29.8 \pm 1.3$</td>
</tr>
<tr>
<td>TSVR (mm Hg/ml/min)</td>
<td>$0.074 \pm 0.02$</td>
<td>$0.083 \pm 0.02^*$</td>
<td>$0.071 \pm 0.02$</td>
</tr>
<tr>
<td>After HF ($n = 6$)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>$133 \pm 19^*$</td>
<td>$138 \pm 15$</td>
<td>$124 \pm 13^*$</td>
</tr>
<tr>
<td>$E_{\text{ES}}$ (mm Hg/ml)</td>
<td>$3.6 \pm 0.7^*$</td>
<td>$7.2 \pm 1.5^*$</td>
<td>$3.6 \pm 0.8^*$</td>
</tr>
<tr>
<td>Time constant of relaxation (ms)</td>
<td>$43.2 \pm 5.4^*$</td>
<td>$34.6 \pm 4.5^*$</td>
<td>$45.2 \pm 8.4$</td>
</tr>
<tr>
<td>TSVR (mm Hg/ml/min)</td>
<td>$0.091 \pm 0.02^*$</td>
<td>$0.072 \pm 0.02^*$</td>
<td>$0.088 \pm 0.02^*$</td>
</tr>
</tbody>
</table>

* $n$, numbers of dogs. Hemodynamic values are mean ± S.D.

† $p < 0.05$, after drug vs. corresponding baseline.

‡ $p < 0.05$, HF baseline vs. normal baseline.

§ $p < 0.05$, HF DOB vs. normal DOB.

HP DOB vs. HF p.o. LS.

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 noninvasive assessments (Hosenpud and Group, 1999; Föder et al., 2003, 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, three incremental dosages of oral LS caused dose-related, significantly increased LV contractility ($E_{\text{ES}}$ and $M_{\text{SV}}$) and decreased time constant of LV relaxation ($\tau$). It is noteworthy that these changes of $E_{\text{ES}}$ and $\tau$ 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 approximately 10 ng/ml are required to generate clear changes in $E_{\text{ES}}$ 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 57033 and 53998, ORG 30029, and CGP 48506), are supported by several prior experimental studies (Hgashiyama et al., 1995; Tachibana et al., 2005). Enhancing the calcium sensitivity of the contractile proteins and phos-
Ca²⁺ found that during the development of HF, the EES/EA ratio reduce SW (Little and Cheng, 1994). In the current study, we LV and arterial system are nearly optimally coupled to pro-

our laboratory have demonstrated that normal-functioning echocardiographic study in severe HF patients.

most recent clinical combined hemodynamic and Doppler stage failing human hearts (Hasenfuss et al., 1998) and by a 

by evidence in vitro indicating that LS accelerates and does contribute to the dose-related enhancements of LV contrac-
tile performance and relaxation. Our findings are supported 

cells and mitochondrial membranes (Yokoshiki et al., 1997a,b; Kaheinen et al., 2001) as well as recent demonstra-
tions in severe HF (Follath et al., 2002) and further encour-

ged positive inotropic action (Edes et al., 1995; Moiseyev et al., 2002; Avgeropoulou et al., 2005), anti-in-
flammatory, and antiapoptotic 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 SW (Little and Cheng, 1994). In the current study, we found that during the development of HF, the EES/Eₐ ratio was reduced, resulting in less than maximal SW. Single oral target doses of 0.025, 0.05, and 0.1 mg/kg LS caused a dosen-related increase in EES but decreased Eₐ, thus causing an increase in the EES/Eₐ ratio with resulting near-maximum SW after HF. Eₐ 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 EES/Eₐ ratio and LV mechanical efficiency after HF. 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 (Ni-

emin 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 because 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 compared with placebo- and/or dobut-
amine-treated patients. On the contrary, two recent large tri-

als (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 i.v. LS and Dobutamine in Normal and HF

Before HF, dobutamine (6 μg/kg/min), i.v. LS (24 μg/kg, followed by 0.2 μg/kg/min), and oral LS (0.05 mg/kg) pro-
duced similar positive inotropic and lusitropic action. Only dobutamine increased HR. After HF, although oral and i.v. LS-induced positive inotropic and lusitropic effects persisted, the chronotropic effect was attenuated. In contrast, dobut-
amine showed blunted positive inotropic and lusitropic actions, but the positive chronotropic effect persisted. In HF, oral and i.v. LS caused similar Cₘₐₓ levels with resultant similar favorable reductions in Vₑₑₑ, Pₑₑₑ, Eₐ, TSVR, and Wₛₑₑₛ, correlating with similar improvement in LV-arterial coupling and LV mechanical efficiency. This important property of LS differentiates LS from (conventional) β 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 encour-

Fig. 5. Examples of comparison of the peak effects of oral LS (0.05 mg/kg) and dobutamine (DOB) (6 μg/kg/min) on LV Pₑₑₑ-Vₑₑₑ relationship in normal and HF states. LV Pₑₑₑ-Vₑₑₑ 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 Pₑₑₑ-Vₑₑₑ relationship (Eₑₑₑ), which were similar to DOB (6 μg/kg/min i.v.). After HF, the cardiac response to DOB was blunted; however, LS-positive inotropy persisted.
aged 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 neurohumoral 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 (Lenfant, 1994; Cheng et al., 1996; Pagel et al., 1997; Bristow, 2000). However, we cannot be certain that our results apply to HF of other causes such as hypertrophic cardiomyopathy. In addition, 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 active 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 venodilation 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.

References


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