Direct Myocardial Effects of Levosimendan in Humans With Left Ventricular Dysfunction

Alteration of Force-Frequency and Relaxation-Frequency Relationships

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Background—Enthusiasm for the development of Ca\textsuperscript{2+} sensitizers as inotropic agents for heart failure has been tempered by reports of impaired relaxation. Levosimendan, which increases myofilament Ca\textsuperscript{2+} sensitivity via Ca\textsuperscript{2+}-dependent binding to troponin C, exerts positive inotropic and lusitropic effects in failing human myocardium in vitro. We sought to determine the direct effects of levosimendan on failing human myocardium in vivo, and in particular whether levosimendan exerts heart rate–dependent effects on systolic or diastolic function.

Methods and Results—Ten patients with left ventricular dysfunction caused by nonischemic dilated cardiomyopathy (mean left ventricular ejection fraction, 27±2%) were instrumented with an infusion catheter in the left main coronary artery, a high-fidelity micromanometer-tipped catheter in the left ventricle, and a bipolar pacing wire in the right atrium. Inotropic (peak \(\text{dP/dt}\)) and lusitropic (\(\text{Tau}\)) responses were assessed during continuous intracoronary drug infusion in sinus rhythm followed by atrial pacing at 20, 40, and 60 beats per minute above the sinus rate. Under control conditions (intracoronary 5% dextrose in water), atrial-pacing tachycardia decreased \(\text{Tau}\) by 13% (\(P<0.05\)), but did not increase \(\text{dP/dt}\). Intracoronary levosimendan (3.75 and 12.5 \(\mu\text{g/min}\) for 15 minutes each) increased \(\text{dP/dt}\) dose-dependently and decreased \(\text{Tau}\) over a range of heart rates, but did not alter the slope of the force-frequency or relaxation-frequency relationship.

Conclusions—Myocardial calcium sensitization with levosimendan exerts mild inotropic and lusitropic effects in humans with left ventricular dysfunction, but does not alter the force-frequency or relaxation-frequency relationship. (Circulation. 2007; 115:1218-1224.)

Key Words: calcium ■ contractility ■ heart failure ■ levosimendan ■ myocardium

Despite optimal medical therapy, patients with chronic left ventricular (LV) systolic dysfunction often require hospital admission for symptoms of worsening congestion and/or systemic hypoperfusion.\textsuperscript{1} Positive inotropic agents currently available for the treatment of decompenated heart failure, which include dobutamine and milrinone, are limited by their tendency to increase heart rate and stimulate arrhythmias. These adverse effects in failing human myocardium are mediated primarily by an increase in intracellular calcium. Calcium-sensitizing agents, which act by directly increasing the sensitivity of the myofilament to calcium, may exert positive inotropic effects without proarrhythmia. The enthusiasm for the development of calcium sensitizers has been tempered, however, by reports of impaired relaxation,\textsuperscript{2} especially under hypoxic conditions or at higher stimulation frequencies, and reduced energy efficiency as a result of increased crossbridge cycling.

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Levosimendan enhances calcium sensitivity of the contractile apparatus via calcium-dependent binding to cardiac troponin C.\textsuperscript{3-5} In skinned fibers from normal guinea pig papillary muscles, levosimendan causes concentration-dependent, direct positive inotropic effects without impairment of relaxation. Hasenfuss et al\textsuperscript{6} studied the effects of levosimendan on failing human myocardium in vitro and demonstrated both positive inotropic and lusitropic effects over a range of twitch frequencies. Furthermore, there was no frequency-dependent rise in diastolic tension with levosimendan. In muscle strips with weak inotropic responses, the increase in intracellular calcium was significantly higher with milrinone than with levosimendan, which suggests different modes of action. In dogs with pacing-induced heart failure, levosimendan improved both systolic and diastolic function without a change in heart rate or myocardial oxygen consumption.\textsuperscript{7} Improve-
ment in systolic function without an increase in myocardial oxygen consumption has also been demonstrated in healthy volunteers and patients with cardiovascular disease. In normal myocardium, an increase in heart rate results in an increase in contractile force, which is reflective of the fact that a positive relationship exits between force and frequency. The force-frequency relationship, originally described in the frog heart more than 120 years ago, has subsequently been confirmed in cardiac muscle strips and isolated ventricular myocytes from most mammals, including humans, and in the intact normal human heart. In contrast, the failing human heart has a force-frequency relationship that is attenuated, flat, or even inverted. The mechanism responsible for the ties in calcium handling that worsen at higher frequencies. Drug infusions have been described previously. A 6F L4 Judkins method used for hemodynamic measurements and intracoronary media, and the research protocol was begun a minimum of 20 years ago. Before the experimental protocol, all subjects underwent routine diagnostic left and right heart catheterization via the femoral approach. Coronary angiography was performed with nonionic contrast media, and the research protocol was begun a minimum of 20 minutes after completion of the diagnostic catheterization. Detailed methods used for hemodynamic measurements and intracoronary drug infusions have been described previously. A 6F L4 Judkins catheter (Cordis, a Johnson & Johnson Company, Miami Lakes, Fla) was advanced from the right femoral artery to the left main coronary ostium for intracoronary drug infusion. A 7F high-fidelity micrometer-tipped pigtail catheter (Millar Instruments, Houston, Tex) was advanced from the left femoral artery and positioned in the LV for measurement of LV pressure. A 5F bipolar pacing wire was advanced from the right femoral vein to the right atrial appendage for atrial pacing. Femoral artery pressure was monitored from a 7F sidearm sheath (Cordis) present in the right femoral artery. After instrumentation, an additional 5000 U of heparin was administered intravenously. Hemodynamic measurements included heart rate, LV systolic pressure (LVSP), LV end-diastolic pressure (LVEDP), and LV pressure measured intracoronary during the fifth minute. Right atrial pacing then initiated at a rate of 20 beats per minute above the baseline sinus rate for 2 minutes, and hemodynamics were measured during the second minute. Atrial pacing was then increased to 40 and 60 bpm above the baseline sinus rate. The pacing protocol was terminated if there was development of atrioventricular block or evidence of myocardial ischemia (eg, angina, ST changes, or increased LVEDP). To reestablish baseline conditions, the pacemaker was turned off, and D5W continued to be infused into the left main coronary artery for at least 5 minutes until all hemodynamics had returned to baseline values (±10%). Levosimendan was then infused into the left main coronary artery at rates of 3.75 and 12.5 μg/min for 15 minutes each. These rates were chosen to achieve intracoronary steady-state levosimendan concentrations of 30 and 100 ng/mL, respectively, with an assumption of a left coronary blood flow rate of 125 mL/min. In patients with acute decompensated heart failure, levosimendan exerted sustained hemodynamic effects when administered intravenously at mean doses that ranged from 0.14 to 0.26 μg/kg per min, and achieved plasma concentrations of ~60 ng/mL and 120 ng/mL, respectively. During the tenth minute at each infusion rate, hemodynamics were recorded in normal sinus rhythm. Right atrial pacing was then performed successively at 20, 40, and 60 bpm above the baseline sinus rate, with hemodynamic measurements and pacing end points as described for the control condition.

Statistical Analysis
The hemodynamic variables of primary interest were LV peak +dP/dt and Tau. Each variable was analyzed separately with analysis of variance with a repeated measures model to assess the effects of treatment and atrial pacing. Each dose of levosimendan was compared with the control by use of the Dunnett-Hsu test. A test for linear dose response was performed with a repeated measures analysis of covariance. All analyses were performed with SAS statistical package version 6 (SAS Institute, Inc, Cary, NC), and data are presented as mean ± SEM. Statistical results were considered significant if the probability of obtaining the results by chance was 0.05 or less. All tests were 2-tailed.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results
Baseline Hemodynamics and Response to Intracoronary Levosimendan in Sinus Rhythm
Baseline hemodynamics revealed chronic heart failure with an average heart rate of 76 bpm, and LVSP and LVEDP of 131 mm Hg and 32 mm Hg, respectively (Table 1). Chronic LV systolic dysfunction was associated with marked impairment in both contractility and isovolumic relaxation with a
mean LV peak $+\frac{dP}{dt}$ of $769\pm 57$ mm Hg/s and Tau of $72\pm 6$ ms, respectively. In sinus rhythm, intracoronary levosimendan at 3.75 and 12.5 $\mu$g/min caused modest dose-dependent increases in LV peak $+\frac{dP}{dt}$ of 4.5$\pm$2.0% and 7.4$\pm$2.8%, respectively ($P<0.05$ versus control for both), despite non-significant reductions in LVEDP (Figure 1). Interpatient variability in the inotropic response to high-dose levosimendan was evidenced by a range in the percent change in LV peak $+\frac{dP}{dt}$ from $-9\%$ to 21% (Figure 2A). Tau was unchanged after low-dose levosimendan but decreased by 8.6$\pm$3.6% after intracoronary levosimendan at 12.5 $\mu$g/min ($P<0.05$ versus control). Interpatient variability in the lusitropic response to high-dose levosimendan was also observed, and the percent change in Tau ranged from $-25\%$ to 15% (Figure 2B). Intracoronary levosimendan had no effect on heart rate or LVSP (Table 1).

**Effect of Intracoronary Levosimendan on the Force-Frequency Relationship**

Under control conditions (intracoronary D5W), atrial pacing to a maximal heart rate of 133$\pm$6 bpm resulted in reductions in LVSP from 131 mm Hg to 119 mm Hg ($P<0.05$) and LVEDP from 32 mm Hg to 22 mm Hg ($P<0.05$) without a change in LV developed pressure (Table 2). Atrial pacing resulted in a modest increase in LV peak $+\frac{dP}{dt}$ at 20 and 40 bpm above the baseline sinus rate, but there was no difference in contractility at the highest pacing rate compared with the baseline sinus rate ($800$ mm Hg/s versus $769$ mm Hg/s, $P=\text{NS}$), consistent with an attenuated force-frequency relationship. Levosimendan at 3.75 and 12.5 $\mu$g/min resulted in mild, dose-dependent, positive inotropic effects during atrial pacing (Table 2, Figure 3). Specifically, LV peak $+\frac{dP}{dt}$ increased by 6% and 8%, respectively, at 20 bpm above the baseline sinus rate and by 6% and 9%, respectively, at 40 bpm above the baseline sinus rate. However, levosimendan had no effect on the slope of the force-frequency relationship (Figure 3).

**Effect of Intracoronary Levosimendan on the Relaxation-Frequency Relationship**

Under control conditions, atrial pacing resulted in a gradual decrease in Tau from 72 ms during baseline sinus rhythm to

![Figure 1](image1.png)

Figure 1. Relationship between changes in LV peak $+\frac{dP}{dt}$ and LVEDP with intracoronary levosimendan (Levo) at 3.75 and 12.5 $\mu$g/min in normal sinus rhythm.

![Figure 2](image2.png)

Figure 2. Positive inotropic and lusitropic responses to intracoronary levosimendan (Levo) at 12.5 $\mu$g/min. Shown are the percent changes in LV peak $+\frac{dP}{dt}$ (A) and Tau (B) for individual numbered subjects in normal sinus rhythm. *$P<0.05$ versus control.

| Table 1. Hemodynamic Response to Intracoronary Levosimendan in Normal Sinus Rhythm |
|------------------------------|-----------------|-----------------|
| Control | Levo at 3.75 $\mu$g/min | Levo at 12.5 $\mu$g/min |
| HR, bpm | 76$\pm$5 | 76$\pm$4 | 76$\pm$4 |
| LVSP, mm Hg | 131$\pm$5 | 134$\pm$5 | 135$\pm$5 |
| LVEDP, mm Hg | 32$\pm$3 | 30$\pm$3 | 29$\pm$3 |
| LVPdev, mm Hg | 100$\pm$5 | 104$\pm$6 | 106$\pm$6$^*$ |
| LV peak $+\frac{dP}{dt}$, mm Hg/s | 769$\pm$57 | 802$\pm$59$^*$ | 826$\pm$62$^*$ |
| Tau, ms | 72$\pm$6 | 72$\pm$7 | 65$\pm$5$^*$ |

Levo indicates levosimendan; HR, heart rate; and LVPdev, left ventricular developed pressure.

*$P<0.05$ vs control.
63 ms at 60 bpm above the baseline sinus rate (P<0.05), consistent with a positive relaxation-frequency relationship (Table 2). Compared with control, low-dose levosimendan had no significant effect on Tau during atrial-pacing tachycardia, whereas levosimendan at 12.5 μg/min resulted in mild positive lusitropic effects over a range of heart rates (Table 2, Figure 4). Specifically, Tau decreased by 14%, 11%, and 11% at 20, 40, and 60 bpm above the baseline sinus rate. However, levosimendan had no effect on the slope of the relaxation-frequency relationship (Figure 4).

Safety
At the intracoronary doses used, levosimendan was well tolerated in all patients without hypotension or proarrhythmia. No patients developed evidence of myocardial ischemia (eg, angina, ST segment changes, or increased LVEDP) during atrial pacing tachycardia.

Discussion
In the present study, we assessed the direct myocardial effects of levosimendan in patients with heart failure by administration of subsystemic doses of drug into the left main coronary artery while heart rate was altered with right atrial pacing. We found that levosimendan exerts modest positive inotropic and lusitropic effects over a range of heart rates, but does not alter the slope of the force-frequency or relaxation-frequency relationship. These novel in vivo data provide the first look at the direct myocardial actions of levosimendan not confounded by systemic vascular effects and extend prior in vitro findings from failing animal and human myocardium.

Inotropic Effects of Levosimendan
Levosimendan is a pyridazinone-dinitrile derivative that enhances calcium sensitivity of the myofilaments via calcium-dependent binding to troponin C.20 Discovered by screening for compounds with high affinity to the troponin complex, levosimendan stabilizes troponin C in a conformation that...
triggers contraction. Multiple laboratory studies have shown that levosimendan exerts positive inotropic effects in both normal and failing myocardium with a median effective dose of $\approx 0.1\ \mu\text{mol/L}$. Subjects in this study had chronic dilated cardiomyopathy with marked impairment in systolic function (average LV peak $+dP/dt$, 769 mm Hg/s). Levosimendan exerted modest dose-dependent effects on $+dP/dt$ over a wide range of heart rates (mean, 76 to 133 bpm), with an average 8% increase from baseline.

In addition to calcium sensitization, levosimendan has other pharmacological properties that may have contributed to the positive inotropic (and lusitropic) effects we observed. Initial in vitro studies demonstrated potent and selective inhibition of phosphodiesterase type III. However, subsequent experiments showed that with lower doses of levosimendan, similar to those used in this study, phosphodiesterase III inhibition does not contribute to positive inotropic effects.

Levosimendan also causes vasodilatation via opening of adenosine triphosphatase-sensitive $K^+$ channels. This effect may contribute to coronary and systemic vasodilation with the intravenous administration of levosimendan. The direct effects of $K^+$-channel opening on myocardial contractility remain unclear, although 1 study in an animal model of diabetic cardiomyopathy suggested a negative inotropic effect.

### Lusitropic Effects of Levosimendan

Unlike other agents that increase calcium sensitization via calcium-independent mechanisms (eg, MCI-154, EMD-57033), levosimendan does not impair myocardial relaxation in vitro. Pagel et al showed that levosimendan had no deleterious effect on myocardial relaxation in normal dogs and improved resting diastolic function in dogs with heart failure. Additional animal data show that levosimendan attenuated the increase in LVEDP and decreased Tau during exercise. In failing and nonfailing human myocardium, Brixius et al demonstrated a positive lusitropic effect of levosimendan, in part mediated by cAMP. Activation of $K^+$ channels also improves relaxation in failing myocardium.

Our in vivo human data show that levosimendan improves both cardiac contractility and relaxation in patients with heart failure. To our knowledge, this is the first catheterization laboratory–based study to assess changes in isovolumic relaxation in response to levosimendan. Previous studies that used intravenous administration of levosimendan have been confounded by systemic effects, which include reduction in LV preload and afterload. Improvement in diastolic function, even if modest, might contribute to favorable effects of levosimendan in patients with acute heart failure regardless of ejection fraction. In acute coronary syndromes, levosimendan improves diastolic function and causes an upward and leftward shift of the pressure-volume relationship that is consistent with improved contractile function of stunned myocardium.

### Interpatient Variability

As previously demonstrated in our laboratory with intravenous and intracoronary dobutamine, we observed significant interpatient variability in the inotropic and lusitropic responses to intracoronary levosimendan in heart failure. In explanted failing human myocardium, Hasenfuss et al also demonstrated wide variability in the inotropic response to levosimendan with the peak increase in twitch tension ranging from $-35\%$ to $+80\%$. One explanation for the interpatient variability that we observed may be varying etiologies of heart failure. However, in the study by Hasenfuss et al, etiology (ischemic versus nonischemic) did not explain the variability in the inotropic response. Other explanations for interpatient variability include differences in duration of heart failure and alteration of myocardial gene expression, variability in phosphorylation of troponin I, which may alter the affinity of levosimendan to troponin C, and differences in cardiac medications, resting hemodynamics, or both.

### Force-Frequency and Relaxation-Frequency Relationships

The force-frequency relationship is attenuated, flat, or inverted in failing myocardium. Notably, the degree to which this relationship is blunted in heart failure correlates with the reduction in peak functional capacity. Underlying cellular and molecular mechanisms are not well understood, but frequency-dependent impairment in calcium handling by the sarcoplasmic reticulum has been implicated. Few investigators have demonstrated correction of the force-frequency abnormality in heart failure. Schwinger et al demonstrated partial reversal of the negative force-frequency relationship in failing human myocardium with low-dose isoprenaline, and similar in vitro effects have been observed with gene transfer of sarcoplasmic reticulum calcium ATPase. Myocyte “recovery” after mechanical circulatory support is also associated with improved force-frequency. Most recently, biventricular pacing was shown to improve the inotropic response to increased heart rates in patients with advanced heart failure and ventricular conduction delay. The effect of chronic neurohormonal blockade (eg, with angiotensin-converting enzyme inhibitors and $\beta$-blockers) on the force-frequency relationship is unknown.

The relaxation-frequency relationship is not as well studied, but may have important functional significance in both normal and failing hearts. We observed a $\approx 13\%$ reduction in Tau with atrial pacing tachycardia in both the control state and after intracoronary levosimendan. Our intact human data
with levosimendan are nearly identical to ex vivo studies in failing human myocardium. This positive lusitropic effect has not been observed with other calcium sensitizers, and may be caused in part by phosphodiesterase III inhibition that leads to activation of protein kinase A and phosphorylation of phospholamban. Without control subjects, we cannot comment on whether the relaxation-frequency relationship in heart failure is preserved; however, we previously demonstrated preservation of the lusitropic response to β-adrenergic receptor stimulation in patients with severe heart failure, and others have shown preserved relaxation-frequency relations in adults and children with myocardial hypertrophy.

**Study Limitations**

By infusing levosimendan directly into the left main coronary artery, we were able to avoid changes in loading conditions that might confound the interpretation of +dP/dt and Tau. In the present study, levosimendan increased +dP/dt despite a nonsignificant reduction in LV filling pressure. We cannot exclude the possibility that levosimendan caused coronary vasodilation and increased myocardial blood flow; however, doses used in our study were 10- to 100-fold lower than those used by Michaels et al. The use of β-blockers was low and this may have affected the response to intracoronary levosimendan; such an interaction was shown previously with dobutamine and enoximone. Last, we cannot comment on the acute cellular actions of levosimendan or the myocardial effects of levosimendan given at higher doses, for longer duration, or during physiological tachycardia (eg, with exercise).

**Conclusions**

In summary, the present study demonstrates that levosimendan exerts positive inotropic and lusitropic effects over a range of heart rates in patients with LV systolic dysfunction. These effects are therefore independent of heart rate and systemic vascular actions of the drug. In addition, levosimendan does not alter the slope of the force-frequency or relaxation-frequency relationship. This hemodynamic profile is consistent with a primary site of action that is distal to calcium handling proteins that are involved in abnormal calcium homeostasis in failing myocardium.

**Sources of Funding**

This work was supported in part by a grant-in-aid from the American Heart Association, Massachusetts Affiliate (to Dr Givertz), an American College of Cardiology/Merck Fellowship Award (to Dr Andreou), and by Orion Pharma, Espoo, Finland.

**Disclosures**

Dr Colucci has received grant support from Abbot for the Randomized Multicenter Evaluation of Intravenous Levosimendan Efficacy (REVIVE) trial.

**References**


**CLINICAL PERSPECTIVE**

Positive inotropic agents currently approved for the treatment of acute decompensated heart failure increase heart rate and stimulate arrhythmias by increasing intracellular calcium. Calcium-sensitizing agents were developed as an alternative and potentially safer means of inotropic support. Enthusiasm was tempered, however, by reports of impaired relaxation and the identification of more complex mechanisms of action that may contribute to adverse effects. Levosimendan increases myocardial calcium sensitivity by binding to troponin C and, in failing myocardium, has been shown to exert positive inotropic and lusitropic effects. In the present study, we demonstrated that subsystemic doses of levosimendan administered directly into the left main coronary artery increased cardiac contractility and relaxation over a range of heart rates dose-dependently, but did not alter the slope of the force-frequency or relaxation-frequency relationship. Although these in vivo mechanistic data do not shed additional light on the clinical controversy that surrounds levosimendan, they are notable in the demonstration of a remarkable consistency with in vitro findings in failing human myocardium, and they emphasize the importance of interpatient variability in hemodynamic responses to vasoactive therapy in heart failure. Additional studies are warranted to determine the functional significance of the heart rate–independent effects of levosimendan on failing myocardium, and to elucidate the variability in gene or protein expression that may contribute to differential patient responses in heart failure. With further advances in the field of pharmacogenomics, variability in the short- and long-term toxicity of cardiotonic agents may also be identified.
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Circulation. 2007;115:1218-1224; originally published online March 5, 2007;
doi: 10.1161/CIRCULATIONAHA.106.668640
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/115/10/1218

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