A dose–response study of levosimendan in a porcine model of acute ischaemic heart failure

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OBJECTIVES: Levosimendan is a novel inotropic agent claimed to improve myocardial contractility by a calcium-sensitizing effect. Our aim was to evaluate dose-dependent effects of levosimendan on left ventricular (LV) contractility and energetic properties in an acute, ischaemic heart failure porcine model.

METHODS: Six pigs were used in an anaesthetized in vivo open-chest model. The time points of measurements were: baseline, after heart failure induction and after dose 1–4 (D1–D4). Heart failure was induced by microembolization of the left coronary artery before infusion of four different doses (D1: 2.5 µg/kg, D2: 10 µg/kg, D3: 40 µg/kg, D4: 80 µg/kg) of levosimendan. Haemodynamics were assessed by the pressure-conductance catheter technique. LV oxygen consumption was calculated from coronary flow measurements and coronary sinus blood gases. Mitochondrial respiration was studied in biopsies of the LV.

RESULTS: Levosimendan had no significant, load-independent effect on contractile force (slope of preload recruitable stroke work was 34 mmHg immediately following failure and 39 (P = 0.406), 42 (P = 0.219), 46 (P = 0.067) and 41 (P = 0.267) at D1–D4), although the more load-dependent contractility indicator of dP/dtmax was slightly increased at dose 4 (P < 0.05). LV energy conversion efficiency (PVA–MVO2 relationship) remained unaltered at all doses. Maximal mitochondrial respiration decreased after induction of failure and remained at an unaltered low level during levosimendan infusion.

CONCLUSIONS: Surprisingly, levosimendan had no significant effect on contractility, energy efficiency and mitochondrial respiration of the LV, in a porcine model of acute heart failure. At high doses, levosimendan induced vasodilatation and increased heart rate and cardiac output.

Keywords: Dose–response study • Haemodynamics • Inotropy • Mechanoenergetics • Mitochondrial respiration • Phosphodiesterase inhibition

INTRODUCTION

Acute heart failure following heart surgery is usually treated by administration of inotropic substances such as beta-adrenergic agonists and phosphodiesterase-inhibiting drugs. Classical inotropes increase the contractile force at the expense of increased oxygen consumption, namely by increasing cyclic adenosine phosphate and the transport of calcium into the cytosol [1]. A new inotropic substance, levosimendan (SIMDAX®, Orion Corporation, Espoo, Finland), was suggested to have a dual mode of action, inducing increased inotropy [2, 3] and vasodilatation at the same time [2, 4]. Levosimendan has been found to increase the heart’s inotropic state by sensitizing the myofilaments to calcium [3, 5, 6]. This mechanism of augmented contractility is proposed to be less oxygen-consuming than the mechanisms by which beta-adrenergic agonists and phosphodiesterase-inhibiting drugs act. However, in vitro studies have shown that when the dose of levosimendan is increased, a phosphodiesterase 3 (PDE3)-inhibitory effect becomes more pronounced [3, 7, 8].

The clinical setting we envisioned when designing the study was acute postcardiotomy generalized myocardial failure due to poor myocardial protection or microembolization (air, ascending aortic atherosclerosis). However, the heart failure model may also be applicable for post-percutaneous coronary intervention heart failure, as microembolization is thought to be an important contributor to this as well.

In order to investigate the effect of levosimendan on haemodynamics, myocardial mechanical work and oxygen consumption, we designed a dose–response study and compared the effects of different doses of levosimendan on the left ventricle (LV) and the vascular system in a pig model of acute ischaemic...
heart failure. In addition, mitochondrial respiration in the myocardial cells and the relative distribution of the total LV energy consumption on mechanical and non-mechanical work were analysed [9]. We hypothesized that when increasing the dose of levosimendan in an acute heart failure model, less oxygen-efficient mechanisms of inotropy (i.e. a PDE3-inhibitory effect) would be activated.

**MATERIALS AND METHODS**

The experimental protocol was approved by the local steering committee of the Norwegian Experimental Animal Board. All animals received humane care in compliance with the European Convention on Animal Care. Six pigs (Sus scrofa domesticus, Norwegian strain) of female gender, weight 30–40 kg, were habituated to the animal facilities over 3–7 days and fasted overnight with free access to water. We used an anaesthetized open-chest pig model, as described earlier [10].

**Anaesthesia and medication**

At the day of the experiment, the animals were pre-medicated with intramuscular injection of ketamine (20 mg/kg) and azaperone (5 mg/kg). Anaesthesia was induced by intravenous injection of ketamine (250 mg), atropine (1 mg), pentobarbital-sodium (250 mg) and fentanyl (250 µg). Throughout the experiment the pigs were given a continuous infusion of fentanyl (0.02 mg/kg/h) and midazolam (0.4 mg/kg/h). After the surgical preparation, the animals were given 2500 IU of heparin to avoid clotting of catheters. To prevent arrhythmias, they also received a bolus of amiodarone (300 mg in 50 ml NaCl 0.9% i.v. over 20 min). A bolus of hexamethonium chloride (600 mg in 50 ml NaCl 0.9% over 20 min i.v.) was given to block autonomic reflex influences on haemodynamics. Circulating volume was maintained throughout the experiment by a continuous infusion of Ringer's acetate. At the end of the experiment, the animals were sacrificed by injection of a lethal dose injection of pentobarbital.

**Surgical instrumentation**

After tracheostomy, the pigs were intubated and mechanically ventilated with 60% oxygen. Ventilator settings were adjusted according to blood-gas analysis (PaCO₂ 4–6 kPa). The jugular veins were catheterized for infusions and measurement of central venous pressure (CVP) and the bladder was drained through a cystostomy. Arterial pressure was continuously measured through a catheter inserted into the descending thoracic aorta from one of the femoral arteries. From the same femoral artery catheter, blood samples were drawn for measurements of blood gas and blood resistivity (ρ (µ)). The heart was exposed through a median sternotomy and the left hemiazygos vein was ligated at its passage through the pericardium. Cardiac output (CO) was measured continuously by means of a transit time (MediStim AS, Oslo, Norway) flow probe (16 mm) on the pulmonary artery. Transit time flow probes were also placed on the left anterior descending (LAD), circumflex (Cx) and right coronary arteries for determination of coronary blood flow. The great cardiac vein was catheterized through the superior caval vein via the coronary sinus so that myocardial venous blood from the LV could be sampled. For preload alterations, a rubber band was looped around the inferior caval vein. Furthermore, to adjust for parallel conductance by the hypertonic saline technique, a catheter was inserted into the main pulmonary trunk through the right ventricular wall. Finally, a dual-field, combined pressure–conductance (CD leymcom, The Netherlands) catheter was advanced to the LV cavity via the left carotid artery. Fluoroscopy confirmed the proper placement of the catheter. Importantly, the animals were stabilized for 30 min before any measurements. The instrumentation described here has earlier been thoroughly described in the literature [10].

**Experimental protocol**

Animals were allowed 30 min of stabilization after surgery. After baseline measurements, the microembolization technique was applied to induce acute LV failure [10]. Polystyrene microspheres with a diameter of 55 µm were dissolved in 0.9% NaCl to a concentration of 1 mg microspheres per milliliter. A catheter was placed in the main stem of the left coronary artery under fluoroscopic guidance. LV failure was induced by repeated injections of 2.5–5.0 mg boluses every 5 min until a stable 30% reduction of CO was achieved. Levosimendan was infused following induction of heart failure in increasing dose as outlined below.

Haemodynamic data were sampled at six time points: baseline values (B), after heart failure induction (F) and after each of the four different dose levels of levosimendan (D1–D4). Myocardial biopsies from the LV were taken to assess the mitochondrial respiration at four time measurements: B, F, D2 and D4.

**Levosimendan infusion**

In general guidelines from Orion Pharma, a loading dose of 6–12 µg/kg of levosimendan infused over 10 min and then a continuous dose of 0.1 µg/kg/min is recommended. The patient response should be assessed, and the infusion rate could be decreased to 0.05 or increased to 0.2 µg/kg/min depending on the clinical response. In our study, levosimendan infusion started immediately after induction of heart failure. The first and the last dose intervals were intended to give a concentration level, respectively, well below and well above the recommended clinical blood concentrations. The second and the third dose intervals aimed at approximating the minimum and maximum blood concentrations used clinically. As T1/2 for levosimendan is about 60 min, approximately 270 min infusion time would be required to achieve steady state. It was found impractical to infuse levosimendan over such a long time period in our open-chest pig model. Instead, we infused the dose necessary to achieve steady state over a 30 min period. Clearance was set to zero and from D2 to D4, the amount of levosimendan given in advance was subtracted from the infusion dose. This resulted in the following regime—D1: 2.5 µg/kg, D2: 10 µg/kg, D3: 40 µg/kg, D4: 80 µg/kg. Each level of levosimendan infusion was kept for 30 min and the recordings were performed in the last 10 min (described above).

**Biopsy sampling**

After surgical preparation and exposure of the heart (at time point B), one biopsy was taken from the LV myocardium in the
area between apex and base (BioPince™ needle, Angiotech Pharmaceuticals, Inc, Vancouver, Canada). Biopsies were put into ice-cold solution S (see below) for further treatment within 10 min. Further in a similar manner, three biopsies were sampled at F, D2 and D4.

Mitochondrial respiration

Mitochondrial respiration was studied in situ in saponin-permeabilized fibres as described by Veksler and colleagues [11] and reviewed in detail recently [12]. Briefly, fibres were gently separated using small forceps (Dumont #5) under binocular microscope in solution S at 4°C, and then permeabilized in the same solution with 50 µg/ml saponin for 30 min at 4°C while shaking. After being rinsed for 10 min in solution S at 4°C and then in solution R at 22°C under shaking, the skinned fibres were transferred to a water-jacketed oxygraphic cell (Strathkelvin Instruments, Glasgow, UK) equipped with a Clark electrode containing 3 ml of solution R. Solutions R and S contained 2.77 mM CaK2EGTA, 7.23 mM K2EGTA (100 nM free Ca2+), 6.56 mM MgCl2 (1 mM free Mg2+), 20 mM taurine, 0.5 mM dithiothreitol, 50 mM potassium–methylene sulphionate (160 mM ionic strength) and 20 mM imidazole (pH 7.1 at 22°C). Solution S also contained 5.7 mM Na2ATP, 15 mM creatine-phosphate, while solution R contained 5 mM glutamate, 2 mM malate, 3 mM phosphate and 2 mg/ml bovine serum albumin. Basal respiration rate (V₀) was measured at 22°C under continuous magnet stirring in the oxygraphic cells. Maximal ADP-stimulated respiration above V₀ was measured by the addition of 2 mM ADP as phosphate acceptor and the maximal respiration rate (V_{max}) was calculated as (V₀ + V_{ADP}).

Conductance catheter technique

Evaluation of intraventricular pressure and volume with the conductance catheter technique has been described in the literature in detail [13]. In principle, a pressure–conductance catheter is placed in the LV. The catheter yields simultaneous real-time intraventricular pressure and volume, thus generating instantaneous pressure–volume loops. The volume is calculated from the time-varying electrical conductance which is linearly proportional to the blood volume in the ventricle. Measurements have to be calibrated by deducting the volume related to conductance of the adjacent myocardium (parallel conductance (Vp)) and a calibration factor called slope factor α that compares the stroke volume (SV) of the conductance catheter to a second, independently measured SV. The commonly used formula for the time-varying LV volume (V(t)) was published by Baan et al. [13]:

\[
V(t) = \left( \frac{1}{\alpha} \right) \left( \frac{L}{\sigma_b} \right) G(t) - Vp
\]

in which G(t) is the sum of the conductances, σ_b is the blood conductivity (the inverse property of blood resistivity ρ) measured by a calibrating cuvette and L is the inter-electrode distance.

Data acquisition and analysis

Conductance catheter data formed the basis for the calculation of haemodynamic indices as described in detail elsewhere [10, 14]. These measurements consisted of arterial blood samples for assessment of blood resistivity (ρ) and haemoglobin (Hb). Parallel conductance was estimated by injection of hypertonic 10% NaCl into the pulmonary artery with simultaneous LV pressure–volume sampling. From the conductance alterations induced by intraventricular hypertonic saline, parallel volume was calculated as described elsewhere [10, 13]. Respiratory influence on haemodynamics was avoided by disconnecting the respirator during file-sampling. In order to assess contractility, pressure–volume data were recorded during transient (minimum 10 s) preload reduction by vena cava occlusion. A series of pressure–volume assessments with simultaneous myocardial oxygen consumption (MVO2) recording were then performed at five different steady-state preload reductions in order to assess the PVA–MVO2 relationship, as described elsewhere [9, 10]. Pressure–volume area (PVA, in mmHg × ml) represents the total mechanical work and MVO2 is the calculated LV oxygen consumption, as described by Suga [9]. The PVA–MVO2 relationship represents the LV energy conversion efficiency. Pressure–volume data were recorded while simultaneous Cx and LAD coronary blood flow were assessed and a blood sample for oxygen saturation measurement was drawn from the coronary sinus. Arterial haemoglobin and oxygen saturation were assessed at uninfluenced preload. In the end, a linear regression of the different points of PVA and MVO2 obtained gave the PVA–MVO2 relationship. The conductance and pressure signals were sampled, digitized and stored in a desktop computer connected to the Leycom Sigma 5 DF (CD Leycom).

LV contractile performance was assessed by preload recruitable stroke work (PRSW) and by the rate of LV pressure increase during isovolaemic contractions (dP/dt_{max}). PRSW is the linear relationship between stroke work and end-diastolic volume recorded during vena cava occlusion recordings [15]. The slope of this relationship (Mw) is a contractility index which is less sensitive to load conditions than dP/dt_{max}. Myocardial diastolic function was assessed by dp/dt_{min} and the end-diastolic pressure–volume relationship (EDPVR). General haemodynamic values, such as CO, CVP, middle artery pressure (MAP), heart rate (HR), etc., were also noted. The assessment of diastolic and systolic function has been explained in detail elsewhere [14, 15].

Statistical analyses

Calculations were performed using a spreadsheet (Microsoft Office Excel 2003). All statistical tests were performed in SPSS 17.0. Variables are presented as mean ± standard deviation (SD). We performed one-Sample Kolmogorov–Smirnov test to assess whether the residuals in the linear mixed model were consistent with a normal distribution for the error term. After having confirmed a normality distribution of a variables’ residual, we used a linear mixed model to calculate the P-value between each levels/measurement times. P-values < 0.05 were considered significant.
RESULTS

Pig body weights and LV weights were 34.3 ± 4.0 kg and 113.3 ± 13.3 g, respectively. General haemodynamic data, ventricular mechanics and LV mechanoenergetics at all measurement times are presented in Tables 1–3. CO, systemic vascular resistance (SVR), HR and Mw at all measurement times and maximal mitochondrial respiration at B, F, D2 and D4 are illustrated in Figs. 1–3. Embolization with microparticles in our model was successful in inducing profound heart failure. This was reflected by a statistically significant reduction in important parameters of myocardial pump function; CO, MAP, stroke work (SW) and Mw. After microembolization and stabilization, infusion of levosimendan produced dose-dependent changes in some of the study parameters. A vasodilatation was experienced, reflected by a significant dose-dependent reduction in SVR and MAP. Ventricular contractility indices (Mw and dp/dtmax) were not in line with each other: Mw was not affected by levosimendan infusion while dp/dtmax improved significantly (at D4). At the lowest dose levels of the levosimendan infusion, only the bEDPVR parameter had any statistical significant change. The maximal mitochondrial respiration was reduced after induction of LV failure and thereafter unchanged throughout the total infusion time.

DISCUSSION

In this study, we investigated dose-dependent effects of levosimendan in an open-chest pig model of acute heart failure. We were unable to detect a true increase in myocardial contractility at any dose level of levosimendan. At a low dose of the agent, only the diastolic parameter bEDPVR was affected. Chronotropy and vasodilatory effects were found at higher dose levels. An increase in CO at the highest dose level (80 µg/kg) of levosimendan infusion was one of our main findings. Furthermore, our results contradict the hypothesis that levosimendan at a high dose uses more energy demanding mechanisms of inotropy than levosimendan at lower doses.

It is challenging to study the effect of levosimendan on myocardial contractility in clinical studies due to concomitant medication with other inotropes and the lack of reliable markers of myocardial contractility under these circumstances. We chose an open-chest pig model to be able to isolate the effects of different doses of levosimendan on the failing LV. The well-validated method of conductance catheter measurements was used. Because levosimendan is often used clinically in the setting of ischaemic heart failure, we induced acute ischaemic heart failure in our experimental animals by microparticle embolization into the main stem of the left coronary artery. This method is thoroughly described and validated previously [10]. Published literature suggests that the haemodynamic effects of levosimendan in pigs are similar to the effects reported in humans. Although one could argue that the chosen model does not resemble acute regional ischaemia, we believe that the model is clinically relevant, because it is similar to postcardiotomy low-output failure as a result of generalized myocardial dysfunction as a result of prolonged aortic cross-clamp time, air or particulate embolization (ascending aortic atherosclerosis). With the main goal of understanding levosimendan’s mechanisms of action further, we calculated and assessed different systolic and diastolic indices and we analysed the relationship between myocardial oxygen consumption and mechanical energy of the LV at sub-therapeutic, therapeutic and supra-therapeutic levels of the agent in this setting. Although a similar experimental model of acute heart failure was described in other studies, the contractility indices of the LV were assessed only at baseline and failure in previous studies [9].

Table 1: General haemodynamic data

<table>
<thead>
<tr>
<th></th>
<th>Baseline 0 µg/kg (failure)</th>
<th>2.5 µg/kg (D1)</th>
<th>10 µg/kg (D2)</th>
<th>40 µg/kg (D3)</th>
<th>80 µg/kg (D4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP</td>
<td>93 ± 15</td>
<td>64 ± 13</td>
<td>62 ± 11</td>
<td>63 ± 11</td>
<td>58 ± 8</td>
</tr>
<tr>
<td>HR</td>
<td>97 ± 17</td>
<td>92 ± 16</td>
<td>88 ± 19</td>
<td>94 ± 21</td>
<td>105 ± 26</td>
</tr>
<tr>
<td>CVP</td>
<td>7 ± 3</td>
<td>8 ± 3</td>
<td>8 ± 2</td>
<td>8 ± 2</td>
<td>8 ± 3</td>
</tr>
<tr>
<td>EDP</td>
<td>23 ± 12</td>
<td>24 ± 14</td>
<td>24 ± 11</td>
<td>22 ± 12</td>
<td>22 ± 10</td>
</tr>
<tr>
<td>SV</td>
<td>44 ± 9</td>
<td>31 ± 6</td>
<td>32 ± 5</td>
<td>37 ± 7</td>
<td>33 ± 7</td>
</tr>
</tbody>
</table>

Values are means ± SD (n = 6); exception is CVP: n = 5). Time points of measurement are baseline (B), failure with 0 µg/kg infusion of levosimendan (F) and four different dose levels of levosimendan infusion: 2.5 µg/kg (D1), 10 µg/kg (D2), 40 µg/kg (D3) and 80 µg/kg (D4). MAP: middle artery pressure (mmHg); HR: heart rate (beats/min); CVP: central venous pressure (mmHg); EDP: end-diastolic pressure (mmHg); SV: stroke volume (ml).

Table 2: Ventricular mechanics

<table>
<thead>
<tr>
<th></th>
<th>Baseline 0 µg/kg (failure)</th>
<th>2.5 µg/kg (D1)</th>
<th>10 µg/kg (D2)</th>
<th>40 µg/kg (D3)</th>
<th>80 µg/kg (D4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>dp/dtmax</td>
<td>1657 ± 459</td>
<td>997 ± 349</td>
<td>1036 ± 278</td>
<td>1090 ± 238</td>
<td>1182 ± 195</td>
</tr>
<tr>
<td>SW</td>
<td>3657 ± 1056</td>
<td>1596 ± 572</td>
<td>1723 ± 441</td>
<td>1756 ± 368</td>
<td>1681 ± 424</td>
</tr>
<tr>
<td>bEDPVR</td>
<td>0.019 ± 0.016</td>
<td>0.032 ± 0.026</td>
<td>0.024 ± 0.017b</td>
<td>0.023 ± 0.014b</td>
<td>0.022 ± 0.018b</td>
</tr>
<tr>
<td>dp/dtmin</td>
<td>1402 ± 389</td>
<td>668 ± 286</td>
<td>738 ± 260</td>
<td>754 ± 200</td>
<td>730 ± 194</td>
</tr>
<tr>
<td>τ</td>
<td>78 ± 90</td>
<td>114 ± 95</td>
<td>114 ± 102</td>
<td>111 ± 102</td>
<td>92 ± 82</td>
</tr>
</tbody>
</table>

Values are means ± SD (n = 6). Time points of measurement are baseline (B), failure with 0 µg/kg infusion of levosimendan (F) and four different dose levels of levosimendan infusion: 2.5 µg/kg (D1), 10 µg/kg (D2), 40 µg/kg (D3) and 80 µg/kg (D4). dp/dtmax denotes peak positive first derivatives of LV pressure per time (mmHg/s); SW, stroke work (mmHg × ml); bEDPVR, slope coefficient (b) of the exponential end-diastolic pressure-volume relationship (EDPVR); dp/dtmin denotes peak negative first derivatives of LV pressure per time (mmHg/s); τ, time constant of isovolaemic relaxation (ms).

*Symbolizes a statistical significant difference (P < 0.05) between values after induction of left ventricular failure (failure (F)) versus baseline.

**Symbolizes a statistical significant difference (P < 0.05) between values at D1–D4 versus failure.
previously [2], no experimental evaluation of the effects of different doses of levosimendan on myocardial contractility in an embolization heart failure model has yet been published.

Following embolization, a 29% reduction in CO and a 31% decrease in MAP compared with baseline values, indicated that heart failure was induced. This was confirmed by impaired systolic function represented by a reduction in dP/dt\text{max} and Mw (both \(P < 0.05\)). Additionally, a 20% reduction in myocardial maximal mitochondrial respiration (\(P < 0.05\)) was found. Changes in diastolic properties after microembolization were demonstrated by decreased LV compliance and decreased isovolaemic relaxation rate (indicated by increased \(b_{\text{EDPVR}}\) and decreased \(dp/dt_{\text{min}}\), both \(P < 0.05\)).

We found a statistically significant reduction in MAP and SVR (both \(P < 0.05\)) after levosimendan infusion in our study.

### Table 3: Left ventricular mechanenergetics

<table>
<thead>
<tr>
<th>PVA–MVO(_2) relationship</th>
<th>0 µg/kg (failure)</th>
<th>2.5 µg/kg (D1)</th>
<th>10 µg/kg (D2)</th>
<th>40 µg/kg (D3)</th>
<th>80 µg/kg (D4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slope</td>
<td>1.4 ± 0.8</td>
<td>1.0 ± 0.6</td>
<td>1.1 ± 0.5</td>
<td>1.2 ± 0.6</td>
<td>1.4 ± 0.6</td>
</tr>
<tr>
<td>(y)-intercept</td>
<td>0.37 ± 0.26</td>
<td>0.63 ± 0.52(\ast)</td>
<td>0.43 ± 0.48</td>
<td>0.45 ± 0.36</td>
<td>0.37 ± 0.33</td>
</tr>
<tr>
<td>(r)</td>
<td>0.79 ± 0.08</td>
<td>0.70 ± 0.29</td>
<td>0.72 ± 0.29</td>
<td>0.75 ± 0.22</td>
<td>0.85 ± 0.12</td>
</tr>
</tbody>
</table>

Values are means ± SD (\(n = 6\)). Time points of measurement are baseline (B), failure with 0 µg/kg infusion of levosimendan (F) and four different dose levels of levosimendan infusion: 2.5 µg/kg (D1), 10 µg/kg (D2), 40 µg/kg (D3) and 80 µg/kg (D4). \(PVA\), pressure–volume area; MVO\(_2\): myocardial oxygen consumptions. \(PVA\)–\(MVO\(_2\) relations are means of linear regression coefficients derived from the sets of MVO\(_2\) and PVA values obtained by several different steps of preload reductions in each experiment. The slope: excess MVO\(_2\); the \(y\)-axis intercept: unloaded MVO\(_2\) (J beat\(^{-1}\) 100 g\(^{-1}\)); \(r\): correlations coefficient.

\(\ast\)Symbolizes a statistical significant difference (\(P < 0.05\)) between values at D1–D4 versus failure.
in line with the literature. Levosimendan is a well-known vaso-
dilator [2, 16] in which the mechanisms have been described
earlier [4].

In accordance with several studies in the literature [2, 17, 18],
we conclude that levosimendan increased CO in our study: CO
was significantly increased at the highest dose level D4 (P < 0.05).
In contrast, SV was not increased at any dose level. This lack
of improvement in SV may possibly be explained by a reduced
filling of the ventricle, as presented by the reduced end-diastolic
pressure (EDP) at D4. In view of this, the increase in CO was an
effect of the increased HR.

Levosimendan has been found to improve cardiac contractility
in several studies [2, 3]. In the present study, we calculated
contractility indexes using the conductance catheter technique:
\( \text{dP/} \frac{\text{d}t}{\text{max}} \) had a statistically significant increase of 26% at D4
compared with F (P < 0.05), while Mw had no significant increase
in any of the infusions intervals compared with F. Increased \( \text{dP/}
\frac{\text{d}t}{\text{max}} \) is also reported in a study with anaesthetized pigs with
ischaemic heart by Tassani et al. [2]. It has to be kept in mind
that an increase in \( \text{dP/} \frac{\text{d}t}{\text{max}} \) may be related to augmented
preload and does not reflect a true increase in contractility in all
circumstances. In our experiments, the most reliable index of
contractility (Mw) was not influenced by levosimendan, thus we
conclude that levosimendan had no effect on contractility in the
setting of acute heart failure in our anaesthetized open-chest pig
model.

Few studies have investigated levosimendan’s influence on
myocardial efficiency, and the results are contrasting and difficult
to interpret. In our study, the slope of the PWA–MVO2 relation-
ship did not change at any dose levels of levosimendan infusion.
In view of this, we conclude that levosimendan did not affect
contractile efficiency at any dose level in our model. In contrast
to the slope, the \( y \)-axis intercept (‘the unloaded MVO2’) of the
linear PWA–MVO2 relationship was changed by the levosimen-
dan infusion. The unloaded MVO2 increased from F to D1 and
somewhat surprisingly decreased at larger dose intervals (all P <
0.05). Our data may suggest that a low dose of levosimendan
given to a failing heart induces an increased energy use for
calcium-handling (EC coupling) and metabolic processes, while
higher doses of levosimendan results in decreased energy
expenditure for these non-mechanical processes. Levosimendan
has been proposed to exert different modes of action at low and
high doses [3, 7, 8]. An increasing degree of PDE-inhibition has
been suggested at higher doses. This would have implied an
increased oxygen waste with increasing doses. This was not
found in our study.

In conclusion, our results contradict the hypothesis that levo-
simendan at a high dose employs more energy demanding
mechanisms of inotropy (e.g. phosphodiesterase inhibition) than
levosimendan at lower dose. Possibly supporting our results,
Ukkonen et al. [16] has presented neutral effects of levosimendan
on LV efficiency in patients with congestive heart failure.
However, material and methods differ a lot between this and
our study. In contrast to our results, Pagel et al. [19] have pre-
sented results suggesting increased myocardial efficiency after
levosimendan infusion. Furthermore, Banfor et al. have pre-
sented no increase in myocardial oxygen consumption of levo-
simendan compared with dobutamine at inotropic concentrations
in dogs [20]. However, these studies differ a lot from our experi-
mental animal setup regarding study design and method of esti-
mating the myocardial efficiency. In contrast to the results by
Pagel et al. suggesting improved metabolic efficiency when
infusing high doses of levosimendan, Müller et al. demonstrated
a significant increase in oxygen consumption related to mechan-
ical work when increasing the dose of levosimendan to supra-
therapeutic levels [17]. Tassani et al. [2] have studied the
efficiency of the myocardial performance after levosimendan
infusion following obstruction of a coronary artery in an anaes-
ethetized open-heart pig model. In the hypoperfused area,
a combination of decreasing power index and increasing lactate
release contradict improved myocardial efficiency. Different
effects of levosimendan within the myocardium (between intact
and hypoperfused myocardium) found by Tassani et al. may
explain why studies including hearts with normal function have
presented results contrasting to studies including hearts with
post-ischaemic function when assessing its efficiency after levo-
simendan infusion [17, 19]. It is possible that the distribution or
ratio of ischaemic versus non-ischaemic myocardium in our study
may have affected our conclusion of the functional effects of
levosimendan.

Only a few investigators have analysed the in vivo effects of
levosimendan on diastolic properties. Investigating the impact
on diastolic properties is important, as calcium sensitizers may
theoretically impair relaxation. In the present study, LV compli-
ance seems to be improved (\( b_{\text{EDPVR}} \) is reduced (P < 0.05)) with
the two lowest doses of levosimendan. This effect is stable
throughout the infusion of gradually higher doses of levosimen-
dan. In contrast, the isovolaemic relaxation rate was at no
infusion-level affected by the levosimendan infusion (both \( \text{dP/}
\frac{\text{d}t}{\text{min}} \) and \( r \) were unchanged). In other studies, it is presented a
somewhat surprising decrease in the time constant of relaxation
after levosimendan infusion [21].

In recent investigations, levosimendan is shown to affect mito-
ochondrial function by means of both preconditioning and post-
conditioning. Opening of ATP-dependent K+ channels is the
mechanism described to yield this cardioprotection. Theoretically,
opening of ATP-dependent K+ channels could affect mitochondrial respiration [22]. This could contribute to
improved cardiac function by levosimendan, as reported in clin-
ical studies. To our knowledge, the present study is the first to
study maximal mitochondrial respiration in biopsies from the LV
after levosimendan infusion in a pig model of acute ischaemic
heart failure. The myocardial maximal mitochondrial respiration
decreased following heart failure induction in our study, similar
to findings of Zoll et al. [23] who describe a 20% reduction of the
maximal respiration in the myocardial mitochondria after 45 min
of ischaemia, induced by left descending coronary artery ligation
in pigs. Our results could thus be expected according to the
mitochondrial structural and functional injuries occurring early
in the process of ischaemia [24]. The decline in mitochondrial
respiration was not improved by levosimendan infusion through-
out the experiment.

A limitation of our study is the use of an acute animal model
which made it necessary to infuse higher doses within a shorter
time-interval than standard clinical doses. It would have been
interesting to investigate the effect of levosimendan following
prolonged infusion. However, we believe that our model is clin-
cially very relevant for the setting of acute unexpected postcar-
diomyopathy myocardial failure. Knowledge on the benefits and
shortcomings of acute levosimendan infusion is important to
guide the clinician through different steps of treatment including
mechanical assist systems.

In view of our results, it may have been beneficial to have a
control group without levosimendan. However, changes in other
parameters such as diastolic values, SVR and CO did indicate effective blood concentrations of levosimendan. Besides, our study was designed primarily as a dose–response study in which we aimed at studying dose-dependent effects of levosimendan. Moreover, the animal model used has been validated extensively in previous studies [10]. Thus, it seems unlikely that a control group would have made any difference to the main findings of the study. Furthermore, we chose not to provide any other inotropic agent or vasopressors because the aim of our research was to study the isolated effects of levosimendan on the cardiovascular system.

We conclude that levosimendan in an anaesthetized open-chest pig model of acute heart failure mainly induced vasodilatation, increased HR and improved CO. Our results did not confirm a true increase in contractility or energy saving effect. Furthermore, our results did not support the hypothesis that a less oxygen-efficient mechanism of inotropy (a PDE3-inhibitory effect) would be activated when increasing the dose of levosimendan.

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