



HELI LEPPIKANGAS

Expanding the Indications
for the Use of Levosimendan

Clinical studies in adult patients
and experiments in pigs



ACADEMIC DISSERTATION

To be presented, with the permission of
the board of School of Medicine of the University of Tampere,
for public discussion in the Jarmo Visakorpi Auditorium,
of the Arvo Building, Lääkärintätkatu 1, Tampere,
on May 27th, 2011, at 12 o'clock.

UNIVERSITY OF TAMPERE

ACADEMIC DISSERTATION

University of Tampere, School of Medicine
Tampere University Hospital, Department of Anaesthesia
Finland

Supervised by

Professor Leena Lindgren
University of Tampere
Finland
Professor Esko Ruokonen
University of Eastern Finland
Finland

Reviewed by

Docent Kai Kiviluoma
University of Oulu
Finland
Docent Mika Valtonen
University of Oulu
Finland

Distribution
Bookshop TAJU
P.O. Box 617
33014 University of Tampere
Finland

Tel. +358 40 190 9800
Fax +358 3 3551 7685
taju@uta.fi
www.uta.fi/taju
<http://granum.uta.fi>

Cover design by
Mikko Reinikka

Acta Universitatis Tamperensis 1607
ISBN 978-951-44-8424-7 (print)
ISSN-L 1455-1616
ISSN 1455-1616

Acta Electronica Universitatis Tamperensis 1067
ISBN 978-951-44-8425-4 (pdf)
ISSN 1456-954X
<http://acta.uta.fi>

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List of original publications

This thesis is based on the following original publications, which are referred to by their Roman numerals in the text, and some unpublished data. These data consist of the mortality of patients in Studies I and II, central venous oxygen saturations in Study I, and splanchnic perfusion measurements in Study II. The publications are reprinted with the kind permission of the copyright holders.

I Leppikangas H, Tenhunen JJ, Lindgren L, Salenius JP, and Ruokonen E. (2008) Effects of levosimendan on indocyanine green plasma disappearance rate and the gastric mucosal-arterial pCO₂ gradient in abdominal aortic aneurysm surgery. *Acta Anaesthesiol Scand* 52: 785-792.

II Leppikangas H, Järvelä K, Sisto T, Maaranen P, Virtanen M, Lehto P, Karlsson S, Kööbi T, and Lindgren L. (2011) Preoperative levosimendan infusion in combined aortic valve and coronary bypass surgery. *Br J Anaesth* 106: 298-304.

III Kurola J, Leppikangas H, Magga J, Lindgren L, Kiviniemi V, Rutanen J, and Ruokonen E. (2010) Effect of levosimendan in experimental verapamil-induced myocardial depression. *Scand J Trauma Resusc Emerg Med* 18: 12.

IV Leppikangas H, Ruokonen E, Rutanen J, Kiviniemi V, Lindgren L, and Kurola J. (2009) Levosimendan as a rescue drug in experimental propranolol-induced myocardial depression: A randomized study. *Ann Emerg Med* 54: 811-817.e1-3.

Abbreviations

ADHF	Acute decompensated heart failure
ANG-II	Angiotensin II
ARF	Acute renal failure
AVR	Aortic valve replacement
BE	Base excess
BP	Blood pressure
cAMP	Cyclic adenylylate monophosphate
CABG	Coronary artery bypass grafting
CI	Cardiac index
CO	Cardiac output
CPB	Cardiopulmonary bypass
CPR	Cardiopulmonary resuscitation
CS	Cardiogenic shock
cTnC	Cardiac troponin C
CVP	Central venous pressure
DO ₂	Systemic oxygen delivery
FA	Atrial fibrillation
HF	Heart failure
HR	Heart rate
ICG-PDR	Indocyanine green plasma disappearance rate
ICG _{WB}	Whole-body impedance cardiography
ICU	Intensive care unit
LVEF	Left ventricle ejection fraction
MAP	Mean arterial pressure
mitoK _{ATP}	Mitochondrial ATP-sensitive potassium
MPAP	Mean pulmonary arterial pressure
NT-proBNP	N-terminal pro-B-type natriuretic peptide
OER	Oxygen extraction ratio

ΔPCO_2	Intramucosal-arterial partial pressure of carbondioxide difference
PCWP	Pulmonary capillary wedge pressure
PDE	Phosphodiesterase inhibitor
PPCM	Peripartum cardiomyopathy
PVRI	Pulmonary vascular resistance index
SV	Stroke volume
SI	Stroke volume index
SVR	Systemic vascular resistance
SVRI	Systemic vascular resistance index
ScvO ₂	Central venous oxygen saturation
SvO ₂	Mixed venous oxygen saturation
VF	Ventricular fibrillation
VO ₂	Systemic oxygen consumption

Abstract

Heart failure (HF) is a significant cause of morbidity and mortality worldwide. It is a leading cause of hospitalization among people over 65 years in Western countries and the prevalence in the general population continues to increase. It has also been estimated that the number of major noncardiac surgical procedures in patients aged 65 years and older will increase by 25% in the next 10-20 years. In addition, the population ageing is at hand. Mortality in HF patients is twofold higher after major noncardiac surgery than in patients with coronary artery disease or the general population. The risk of hospital readmission is also higher in patients with HF undergoing common surgical procedures than in other patients. Attempts to reduce the adverse events have not been successful. Therefore, effective treatments for acute HF are needed.

Levosimendan is a fairly new drug developed for the treatment of acutely decompensated heart failure (ADHF). The mechanism of action and current literature suggest that the therapeutic potential of levosimendan may not be limited only to ADHF patients. The purpose of this doctoral thesis was to test new potential clinical indications for levosimendan and to evaluate its haemodynamic effects on systemic and regional blood flow in major surgery.

In Study I, 20 abdominal aortic aneurysm surgery patients received intraoperatively either levosimendan or placebo. The gastric mucosal – arterial pCO₂ gradient measured by an automatic gas tonometry was lower in levosimendan treated patients than in the placebo group. No statistically significant difference could be detected in the total splanchnic blood flow between the groups measured by indocyanine green plasma disappearance rate (ICG-PDR).

In Study II, 24 cardiac surgery patients were randomized to receive levosimendan or placebo one day before surgery. In cardiac surgery patients, the cardiac index (CI) and stroke volume index were higher in the levosimendan group throughout the four-day postoperative period.

In Study III, 12 pigs were intoxicated with verapamil and treated with either levosimendan or placebo. Survival was better in the levosimendan treated animals but there was no difference in the haemodynamic performance between the study groups.

In Study IV, severe propranolol intoxication was induced in 24 pigs. The animals received either levosimendan, dobutamine or placebo. The haemodynamic variables improved and survival was better in the levosimendan group than in the dobutamine and placebo groups.

In conclusion, levosimendan favours gastric perfusion but appears not to have a major effect in total splanchnic perfusion. In cardiac surgery patients, the improvement in cardiac function lasted for four postoperative days in levosimendan treated patients. Levosimendan improved survival in the animal models of verapamil and propranolol intoxication and may provide a feasible alternative to therapy in severe β - blocker intoxication.

Tiivistelmä

Sydämen vajaatoiminta johtaa huomattavaan sairastavuuteen ja kuolleisuuteen maailmanlaajuisesti. Länsimaissa se on yleisin sairaalahoidon syy yli 65- vuotiaille ja sen ilmaantuvuus jatkaa edelleen kasvuaan. Vanhusten määrä lisääntyy ja heillä laajan muun kuin sydänkirurgian on arvioitu lisääntyvän 25% seuraavan 10 - 20 vuoden aikana. Sydämen vajaatoimintaa sairastavien potilaiden kuolleisuus laajassa ei-sydänkirurgiassa on kaksinkertainen verrattuna potilaisiin yleensä tai sepelvaltimotautia sairastaviin potilaisiin. Lisäksi sairaalaan takaisin joutumisen riski on huomattavasti kohonnut. Yritykset vähentää näitä haittatapahtumia eivät ole olleet tuloksekkaita. Näin ollen tehokkaita sydämen vajaatoiminnan hoitoja tarvitaan.

Sairalahoidossa dobutamiini ja milrinoni ovat tavallisimmin käytettyjä suonensisäisesti annosteltavia lääkkeitä sydämen vajaatoimintaan. Näiden cAMP:n kautta vaikuttavien lääkkeiden perusvaikutusmekanismi eli solun sisäisen kalsiumvirran lisääntyminen saattaa altistaa rytmihäiriöille ja lisätä sydämen energiankulutusta.

Levosimendaani on viimeisin kliiniseen käyttöön tullut, Suomessa kehitetty kalsiumherkistäjä, joka lisää sydämen supistusvireyttä lisäämättä solun sisäisen kalsiumin määrää tai sydämen energiakulutusta. Levosimendaanilla on lisäksi laskimoita ja valtimoita sekä sepelvaltimoita laajentava vaikutus. Suomessa levosimendaanilla on tällä hetkellä myyntilupa äkillisesti pahentuneen kroonisen sydämen vajaatoiminnan hoitoon. Levosimendaanin vaikutusmekanismin perusteella voi olettaa, että sen käyttöä muissakin sydämen pumppausvajausta aiheuttavissa tautitiloissa kannattaa tutkia.

Väitöskirjatutkimuksen tarkoituksena oli selvittää, olisiko levosimendaanista hyötyä seuraavissa tilanteissa: 1) potilailla, joilta leikataan vatsa-aortan aneurysma tai 2) korjataan aorttaläppä sepelvaltimoiden ohitusleikkauksen yhteydessä ja kokeellisissa eläinkoetutkimuksissa, jossa aiheutetaan sioille 3) vaikea kalsiumestäjä- tai 4) beetasalpaajamyrkytys.

Osatyössä I 20 vatsa-aortan aneurysman vuoksi leikattavaa potilasta sai leikkauksen aikana joko lumelääkettä tai levosimendaania. Mahalaukun limakalvon ja arteriaveren hiilidioksidigradientti oli automaattisella kaasutonometrillä mitattuna alhaisempi levosimendaanilla hoidetuilla potilailla lumelääkeryhmään verrattuna. Koko suoliston alueen veren virtauksessa ei pystytty havaitsemaan eroa ryhmien välillä, kun mittaus suoritettiin indosyaniini vihreän eliminaatioon perustuvalla menetelmällä.

Osatyössä II 24 sydänleikkauspotilasta satunnaistettiin saamaan joko levosimendaania tai lumelääkettä leikkausta edeltävänä päivänä. Sydämen minuuttivirtaus- ja iskuilavuus indeksit olivat korkeammat levosimendaania saaneilla potilailla lumelääkettä saaneisiin potilaisiin verrattuna neljän leikkauksen jälkeisen vuorokauden ajan koko kehon impedanssi kardiografialla mitattuna.

Osatyössä III 12 sikaa saivat yliannoksen verapamiilia ja ne hoidettiin joko levosimendaanilla tai lumelääkkeellä. Kuolleisuus oli levosimendaaniryhmässä lumelääkeryhmää pienempi, mutta pumppaustuloksissa ei todettu ryhmien välistä eroa.

Osatyössä IV vakava propranololiyliannos aiheutettiin 24 sialle. Siat hoidettiin joko levosimendaanilla, dobutamiinilla tai lumelääkkeellä. Sekä pumppausfunktio että selviytyminen olivat levosimendaaniryhmässä parempia kuin dobutamiini- ja lumelääkeryhmissä.

Väitöskirjatyön päätelminä voidaan todeta, että levosimendaanilla voi olla positiivisia vaikutuksia mahalaukun seinämän verenkiertoon. Koko suoliston alueen verenkiertoa parantavaa vaikutusta ei havaittu. Vuorokautta ennen sydänleikkausta annosteltu levosimendaani parantaa sydämen pumppaustoimintaa neljän leikkauksen jälkeisen päivän ajan. Lisäksi havaittiin, että levosimendaani saattaa parantaa selviytymistä kokeellisessa kalsiumsalpaajamyrkytyksessä. Beetasalpaaja myrkytyksessä levosimendaani näytti parantavan sekä sydämen pumppausvoimaa että selviytymistä ja se voi olla uusi vaihtoehto vaikeiden myrkytysten hoidossa.

1. Introduction

Heart failure (HF) is a significant cause of morbidity and mortality worldwide and is a leading cause of hospitalization among people over 65 years in Western countries (Fonarow, 2008). It has been estimated that the number of major noncardiac surgical procedures in patients aged 65 years and older will increase by 25% in the next 10-20 years. In addition, the share of elderly population will grow and the prevalence of HF in the general population will continue to increase (Hammill et al., 2008). Therefore, effective treatments for acute HF are in demand.

Mortality in HF patients is twofold higher after major noncardiac surgery than in patients with coronary artery disease or general population (Hernandez et al., 2004). The risk of hospital readmission is also higher in patients with HF undergoing common surgical procedures than in other patients; the readmission rate was 17.1% in HF patients compared to 10.8% and 8.1% in coronary artery disease patients and in the comparison group, respectively (Hammill et al., 2008). Attempts to reduce the adverse events have not been successful (POISE Study Group et al., 2008; Sandham et al., 2003).

Several randomized controlled trials have shown improved morbidity and mortality in high-risk surgical patients with perioperative optimisation of haemodynamics (Boyd et al., 1993; Wilson et al., 1999; Shoemaker et al., 1988). Optimisation of cardiac output (CO) requires fluids and inotrope therapy to increase cardiac contractility. Oxygen delivery is dependent on the amount of oxygen in the blood and the CO. Central venous oxygen saturation (ScvO₂) and mixed venous oxygen saturation (SvO₂) have been proposed as indicators of the oxygen supply/demand relationship. Increasing oxygen delivery to achieve normal SvO₂ values during the postoperative period after cardiac surgery can shorten the length of hospital stay (Pölonen et al., 2000). In patients with severe sepsis and septic shock, early ScvO₂- driven haemodynamic treatment was found to reduce mortality (Rivers et al., 2001). Inotropic agents have different effects on circulation to the gut

(Smithies et al., 1994; Meier-Hellmann et al., 1997), which may possibly affect postoperative morbidity (Mythen and Webb, 1994).

Continuous or intermittent intravenous dobutamine or milrinone are commonly used as adjuvant therapy for heart insufficiency. These inotropic agents increase myocardial contractility through different pathways. Dobutamine increases intracellular cyclic adenylylate monophosphate (cAMP) levels by beta-adrenergic-mediated stimulation, which in turn induces an increase in intracellular calcium (Parissis et al., 2007). However, in a large observational trial dobutamine was shown to be of limited benefit and regarding quality of life and survival may even be harmful to patients with advanced HF (O'Connor et al., 1999). Phosphodiesterase (PDE) inhibitors, such as milrinone, increase the cAMP by the selective inhibition of PDE III, the enzyme that catalyses the breakdown of cAMP (Parissis et al., 2007). Milrinone has likewise not lived up to its initial expectations. Chronic HF patients on milrinone treatment had a significantly higher rate of hypotensive episodes requiring intervention and a higher rate of new atrial arrhythmias (Cuffe et al., 2002). One possible explanation for the poor safety record of traditional inotropic agents is that they act through mechanisms that increase the free calcium in myocardial cells. The risk of calcium overload and arrhythmia is thus intrinsic to their mechanism of action (Lehtonen and Pöder, 2007).

Levosimendan is a fairly new calcium-sensitizer that enhances the contractile force of the myocardium without increasing intracellular calcium concentration (Haikala et al., 1995). The opening of ATP-sensitive potassium channels in vascular smooth cells causes vasodilation in the systemic and pulmonary vasculature (Yildiz, 2007). Levosimendan has also been demonstrated to open mitochondrial ATP-sensitive potassium (mitoK_{ATP} channels) and to be effective in protecting the heart against ischaemic / reperfusion injury (du Toit et al., 2008) and to improve the myocardial function of stunned myocardium (Sonntag et al., 2004). The presence of levosimendan's long acting active metabolite OR-1896 has an important role for a clinician. When levosimendan is used, the haemodynamic effects may be seen several days after stopping the infusion (Lilleberg et al., 2007). Dobutamine and milrinone do not have a long lasting active metabolite. The effects of these drugs disappear logically after stopping the infusion according to the elimination half-life.

Levosimendan is indicated for intravenous use in hospitalized patients with ADHF. However, critically ill patients and patients undergoing major surgery often

need inotropic support. In 2009 at Tampere University Hospital, 23% of the cardiac surgery patients needed postoperative inotropic support in the form of dobutamine or milrinone. For critically ill patients this number was 8% (unpublished data). Bearing in mind the disadvantageous effects of conventional drugs dobutamine and milrinone, an alternative therapy could have beneficial effects. However, levosimendan has properties that make it demanding to use in the intensive care setting and in surgical patients. The positive inotropic effects start slowly and are long lasting compared to dobutamine, while the profound vasodilatory effects often require coadministering of vasoconstrictors to counteract hypotension.

The aim of the work reported in this thesis was to find out whether levosimendan could have advantageous properties in the following situations: in patients undergoing abdominal aortic surgery or aortic valve replacement together with coronary artery bypass grafting and in an experimental setting of calcium channel and beta blocker intoxication.

2. Review of the literature

2.1. Levosimendan

2.1.1. Chemistry

Levosimendan [(R) - [[4-(1,4,5,6-tetrahydro-4-methyl-6-oxo-3-pyridazinyl) phenyl]hydrazono] propanedinitrile] belongs to a class of calcium sensitisers. The structural formula is presented in Figure 1. Levosimendan is a moderately lipophilic compound with a molecular weight of 280.3D. It is a weak acid with pK_a 6.3. Levosimendan is commercially available as a powder and must be diluted prior to use with 5% glucose in water to achieve a levosimendan concentrations of 0.025mg/ml or 0.05mg/ml. Levosimendan is administered as an intravenous infusion.

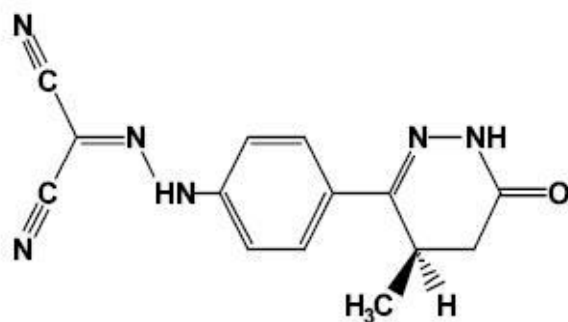


Figure 1. The molecular formula of levosimendan (Pollesello et al., 1994).

2.1.2. Mechanism of action

Levosimendan has three clinically relevant key mechanisms of action: 1) calcium sensitisation improves cardiac contractility; 2) opening of ATP-sensitive potassium channels in vascular smooth cells causes vasodilation and improves oxygen supply

to the myocardium and 3) opening of mitochondrial ATP-sensitive potassium channels protects the heart against ischaemia-reperfusion injury (Figure 2).

2.1.2.1. Calcium sensitisation

The heart contracts at calcium binding to cardiac troponin C (cTnC) and relaxes when calcium dissociates from cTnC. Levosimendan binds to the calcium-saturated cTnC and stabilizes the troponin molecule by prolonging its effects on the contractile proteins (Pollesello et al., 1994). During systole the intracellular calcium concentration is increased and during diastole decreased. The formation of cTnC – levosimendan complex is calcium dependent. This means that levosimendan acts only during systole but does not impair diastolic relaxation (Haikala et al., 1995). Levosimendan has been shown to increase contractility even in a failing human heart where the increase intracellular calcium is modest (Hasenfuss et al., 1998). The cardiac contractility increases without increased oxygen consumption (Nieminen et al., 2009) and with low risk of arrhythmias (Du Toit et al., 1999).

2.1.2.2. Opening of ATP- sensitive potassium channels in vascular smooth cells

The vasodilative mechanism of levosimendan is mainly explained by the opening of ATP-sensitive potassium channels in small resistance vessels and Ca²⁺- activated and voltage- dependent potassium channels in large conductance vessels (Toller and Stranz, 2006). The vasodilation has been demonstrated in both arterial (Yokoshiki et al., 1997a) and venous (Pataricza et al., 2000) vascular beds, in the coronary arteries (Kaheinen et al., 2001) and in the ventricular myocytes (Yokoshiki et al., 1997b). The vasodilation reduces preload and afterload and thereby improves cardiac performance (Harkin et al., 1995), increases coronary blood flow (Lilleberg et al., 1998) and has an anti-ischaemic effect (Kersten et al., 2000). The vasodilatory effect of the active metabolite of levosimendan, OR-1896, has been shown to be comparable with the effects of the parent drug (Banfor et al., 2008). All these effects together may contribute an increase in myocardial contractility aside from those resulting from the calcium sensitisation effect of levosimendan. The duration of vasodilatory effect is long-lasting because of the formation of metabolite OR-1896.

2.1.2.3. Opening of mitochondrial ATP-sensitive potassium channels

The preconditioning is described as a phenomenon in the heart in which a short ischemia will protect the heart from a prolonged ischemic episode. The recent pharmacologic and molecular biological studies suggest that the activation of mitoK_{ATP} channels are responsible for this protective mechanism (Grover and Garlid, 2000). Levosimendan has been demonstrated to open mitoK_{ATP} channels and to be effective in protecting the heart against ischemic / reperfusion injury (du Toit et al., 2008) and improve myocardial function of stunned myocardium (Sonntag et al., 2004).

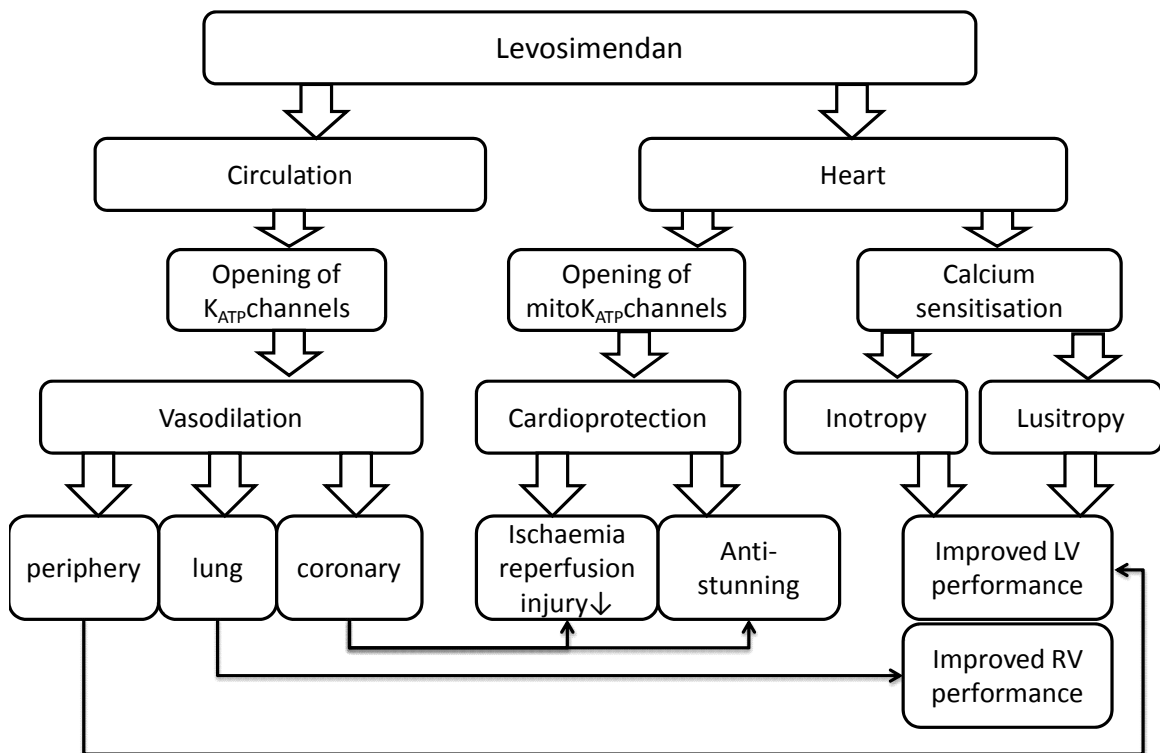


Figure 2. Effects of levosimendan. Modified from the original publication of Pinto et al. (Pinto et al., 2008).

2.1.2.4. Additional mechanism of action

There is also some evidence for levosimendan having immunomodulatory and anti-apoptotic effects. Clinical data in decompensated HF patients demonstrates that

levosimendan seems to decrease pro-inflammatory markers like interleukin-6, C reactive protein, tumour necrosis factor- α , and soluble adhesion molecules (Parissis et al., 2006; Parissis et al., 2008; Adamopoulos et al., 2006). These mechanisms may represent an additional mechanism of action that influences the immune response of critically ill patients.

Furthermore, the role of PDE-III inhibition cannot be excluded. Based on some experimental studies, PDE-III inhibition may contribute to the positive inotropic and lusitropic effect of levosimendan (Boknik et al., 1997; Choi et al., 2010). However, PDE-III inhibition occurs at doses higher than those clinically recommended.

2.1.3. Pharmacokinetics and dosage

Levosimendan is mainly eliminated by metabolism in the liver and intestine. The main pathway is conjugation with glutathione to inactive metabolites. Approximately 5% of the total levosimendan dose is reduced in the intestine to inactive metabolite OR-1855. This intermediate metabolite is further acetylated to the active metabolite, OR-1896 (Antila et al., 1999). Levosimendan is excreted as conjugates via the urine and faeces (Sandell et al., 1995). Levosimendan metabolism is illustrated in Figure 3 (Lehtonen et al., 2004).

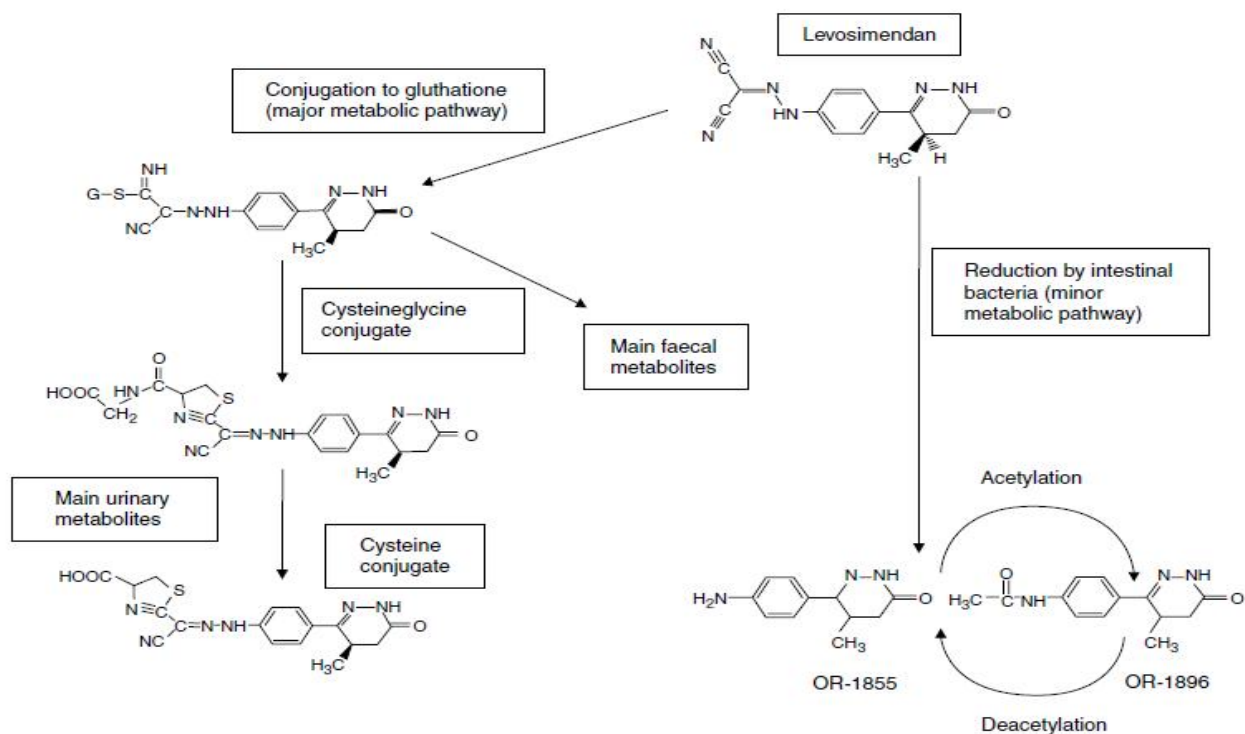


Figure 3. Metabolism of levosimendan. Levosimendan is eliminated mainly as conjugates that are approximately equally excreted into urine and faeces. These main metabolites are inactive. The reduction metabolites, OR-1855 and OR-1896, are formed by intestinal microflora. The OR-1896 has haemodynamic and pharmacologic properties similar to those of levosimendan. Adopted from Lehtonen et al., 2004 with permission.

The haemodynamic effects are a combination of the acute effects of levosimendan itself and the long-lasting effects of OR-1896. The metabolite OR-1896 has been shown to have haemodynamic and pharmacologic properties similar to those of the parent drug in an experimental setting in rats (Segreti et al., 2008) and in dogs (Banfor et al., 2008). Levosimendan is rapidly absorbed and eliminated from the circulation when the infusion is discontinued and the metabolites of levosimendan are formed slowly. In heart failure population the peak level of OR-1896 has been observed at 2-4 days (Lilleberg et al., 2007) (Figure 4).

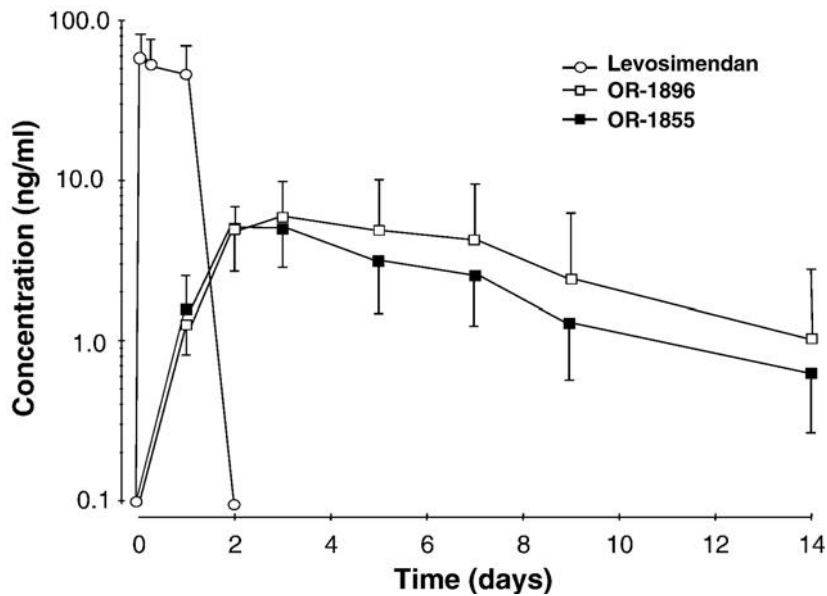


Figure 4. Mean concentrations of levosimendan and its metabolites after a 24-hour infusion in heart failure patients. Adopted from Lillerberg et al., 2007 with permission.

Several interaction studies have been performed using levosimendan. Studies on levosimendan combined with captopril, isosorbide-5-mononitrate and carvedilol have revealed no significant haemodynamic or pharmacokinetic interactions (Antila et al., 1996; Sundberg and Lehtonen, 2000; Lehtonen and Sundberg, 2002). Cytochrome-P450 seems not to be involved in the metabolism of levosimendan based on interaction studies with itraconazole or warfarin (Antila et al., 2000; Antila et al., 1998). The clinical trials report no serious interactions with routine drugs used in HF, such as beta-blockers, digoxin or furosemide (Mebazaa et al., 2007).

The recommendation for the treatment of ADHF is an initial loading dose of 6-12 $\mu\text{g}/\text{kg}$ infused over 10 minutes followed by a continuous infusion of 0.1 $\mu\text{g}/\text{kg}/\text{min}$ for 24 hours. However, according to the results of recent clinical trials it may be advantageous to restrict the treatment to the infusion to avoid possible hypotension and tachycardia (Mebazaa et al., 2007). The dose should be reduced in patients with severe renal insufficiency because of 1.5-fold prolonged elimination of the metabolite OR-1896 compared to healthy subjects (Puttonen et al., 2007). In patients with moderate hepatic impairment the elimination of OR-1896 was also prolonged 1.5-fold but the pharmacokinetics of the parent drug was unaltered. Therefore dosage adjustments of levosimendan may not be required in subjects with impaired hepatic function (Puttonen et al., 2008). Gender, age or race does not affect

the pharmacokinetics of levosimendan when adjusted for body weight (Antila et al., 2007). Levosimendan is equally effective in fast and slow acetylators (Antila et al., 2004).

2.1.4. Adverse events

Levosimendan is generally well tolerated in patients with severe HF. Common side effects are arterial hypotension and headache, which are mainly related to vasodilation. High preinfusion bolus administration and flow rate of levosimendan infusion especially are related to hypotension and headache (Moiseyev et al., 2002).

The RUSSLAN study (Moiseyev et al., 2002) evaluated the safety and efficacy of levosimendan in four different dosages (loading dose of 6-24 μ g/kg + infusion 0.1- 0.4 μ g/kg/min for 6 hrs). When administered at the highest dose rate, levosimendan increased the incidence of hypotension and / or ischaemia (19% in the levosimendan group vs. 11% in the placebo group). At the three lower dose rates, which are the same or more than recommended in clinical practice, levosimendan had no substantial effect on these endpoints.

In the LIDO study (Follath et al., 2002), angina pectoris, chest pain or myocardial ischaemia were less frequent in the levosimendan group compared to dobutamine group. The same trend was seen in the heart rate (HR) and rhythm disturbances. This indicates that levosimendan is well tolerated in severe low output HF patients causing fewer cardiac adverse events than dobutamine.

In the SURVIVE study (Mebazaa et al., 2007), levosimendan-treated patients were less likely to experience cardiac failure than dobutamine- treated patients (12.3% vs.17%, $p=0.02$). On the contrary, the levosimendan-treated patients were more likely to experience atrial fibrillation, hypokalaemia and headache during the 31 days following study drug administration. There was no difference in frequency of hypotension between the study groups. Levosimendan did not significantly reduce all-cause mortality at 180 days.

In the REVIVE-II study, the patients treated with levosimendan experienced more vasodilatory adverse events, such as hypotension and headache, than patients

treated with placebo. However, these results have not been published in peer reviewed forums, the results are provided on the REVIVE II Final Study Report which is available at Clinicalstudyresults.org, which is a database maintained by the pharmaceutical research and manufactures of America.

2.2. Clinical use of levosimendan

The marketing authorisation for levosimendan was first granted in 2000 in Sweden. In Finland levosimendan has been marketed since 2001. In 2009 it is available in 47 countries worldwide and over 400,000 patients have received levosimendan in everyday clinical practice (Product Monograph SIMDAX[®] 2010, Orion Corporation, Espoo, Finland).

2.2.1. Use in acute heart failure

According to the present guidelines of the European Society of Cardiology inotropic agents should be administered to ADHF patients with low systolic blood pressure or low measured CI with signs of organ hypoperfusion or congestion. Levosimendan is recommended for ADHF patients when the treatment with traditional inotropes or vasodilators is insufficient. It is an alternative inotrope for patients treated with beta blockers. The bolus dose is not recommended if systolic blood pressure is below 100 mmHg (European Society of Cardiology. Heart Failure Association of the ESC (HFA). European Society of Intensive Care Medicine (ESICM). Dickstein et al., 2008).

The effects of levosimendan on overall haemodynamics and mortality in major studies including over 100 patients presenting with acute severe HF are summarized in Table 1. The meta-analysis that included 19 studies and 3,650 patients found that levosimendan, when compared to a placebo, was associated with non-significant reduction in mortality, significant improvements in several haemodynamic parameters and reduction in serum B-type natriuretic peptide (NT-proBNP) (Delaney et al., 2010). When levosimendan was compared with dobutamine, levosimendan was associated with better haemodynamics and survival. If this

difference is due to increased mortality with the use of dobutamine or reduction in mortality with the use of levosimendan warrants further study.

Study	Design	N (total /LS)	Comparator	Levosimendan dose	Effect on primary end point	Prespecified follow-up time	Main effect on mortality
European dose finding study ¹	Randomized, double blind, parallel-group (fixed dose)	151/95	Placebo / dobutamine	Bolus 3-36µg/kg + infusion 0.5, 0.1, 0.2, 0.4 or 0.6µg/kg/min for 24 hrs	Produces favourable dose-dependent haemodynamic effects, is well tolerated	9 days	No effect
US dose escalation and withdrawal study ²	Randomized, double blind, parallel group (forced uptitration + withdrawal)	146/98	Placebo	Repeated bolus of 6µg/kg and infusion of 0.1-0.4µg/kg/min for 24 or 48 hrs	Rapid dose-dependent haemodynamic improvement, which maintained for at least 24 hrs after discontinuation of a 24-hour infusion, no tolerance during 48– hour infusion	14 days	No effect
LIDO ³	Randomized, double blind, parallel group, double dummy	203/103	Dobutamine	Bolus 24µg/kg + infusion 0.1-0.2µg/kg/min versus dobutamine 5 -10µg/kg/min for 24 hrs	Haemodynamic improvement was more effective with levosimendan	31 days (mortality at 180 days after code was broken)	Favours levosimendan: after 180 days: levosimendan 26%, dobutamine 38% (p=0.03)
RUSSLAN ⁴	Randomized, double blind, parallel group	504/402	Placebo	Bolus 6, 12 or 24µg/kg + infusion 0.1, 0.2 or 0.4µg/kg/min for 6 hrs	Levosimendan is safe (did not induce hypotension or ischaemia)	14 days (mortality at 180 days after code was broken)	Favours levosimendan: after 180 days: levosimendan: 22.6%, placebo: 31.4% (p=0.05)

CASINO ⁵	Randomized	299/100	Placebo / dobutamine	Bolus 16µg/kg + infusion 0.2µg/kg/min versus placebo / dobutamine 10µg/kg/min for 24 hrs	Mortality benefit at 1 and 6 months	1 year (study was stopped prematurely due to mortality benefit in favour of levosimendan)	Favours levosimendan: 6 month mortality: levosimendan: 12%, dobutamine: 30%, placebo: 19% (p=0.0001 vs. dobutamine and p=0.04 vs. placebo)
REVIVE II ⁶	Randomized, parallel-group (fixed dose)	600/299	Placebo	Bolus 6-12µg/kg + infusion 0.1-0.2µg/kg/min versus placebo for 24 hrs	Modest improvement in the symptoms of heart failure	90 days	No effect
SURVIVE ⁷	Randomized, double-blind, parallel-group	1327/664	Dobutamine	Bolus 12µg/kg + infusion 0.1-0.2µg/kg/min versus dobutamine 5-40µg/kg/m for 24 hrs	No significant effect on mortality at 180 days	180 days	No effect
PORTLAND ⁸	Non-randomized, prospective	129/129	None	Optional loading dose 12µg/kg + infusion 0.05-0.2µg/kg/min for 24 hours	Levosimendan was safe and effective (even without invasive monitoring for majority of patients)	6 months	Mortality was 29% (similar to the levosimendan arm in LIDO)

Table 1. Major studies on levosimendan. Modified from (Lehtonen and Pöder, 2007). ¹ (Nieminen et al., 2000), ² (Slawsky et al., 2000), ³ (Follath et al., 2002), ⁴(Moiseyev et al., 2002), ⁵(Cleland et al., 2004), ⁶ (Cleland et al., 2006), ⁷(Mebazaa et al., 2007), ⁸ (Silva-Cardoso et al., 2009)

2.3. Additional preclinical and clinical use

2.3.1. Cardiac surgery

Cardiopulmonary bypass (CPB) may cause myocardial stunning leading to postoperative left ventricular dysfunction (Chang et al., 2002). Pharmacologic support in the form of vasoactive and inotropic therapy is often required for weaning from cardiopulmonary bypass or increasing tissue perfusion in the perioperative period (Eagle et al., 2004). Maintaining adequate cardiovascular function is essential for sufficient oxygen delivery. A protocolized care aiming at adequate oxygen delivery during the immediate postoperative period after cardiac surgery can decrease morbidity and therefore reduce the length of hospital stay (Pölonen et al., 2000).

A complicated weaning from CBP may lead to myocardial damage, end-organ failure due to impaired organ perfusion and neurologic complications (Murphy and Angelini, 2004). The effect of prophylactic levosimendan administration on weaning and early recovery after CPB was studied in randomized, double-blind study of 60 patients with 3-vessel coronary disease and left ventricular ejection fraction (LVEF) of less than 0.50 (Eriksson et al., 2009). Levosimendan administration (12µg/kg bolus + 0.2µg/kg/min infusion for 24 hours) was started immediately after induction of anaesthesia. Primary weaning was successful in 22 patients (73%) in the levosimendan group and in 10 patients (33%) in the placebo group (p=0.002). Levosimendan treatment was also associated with lower lactate levels, which may indicate improved tissue oxygenation.

Levosimendan has been demonstrated to have beneficial effects on postoperative conditions. One hundred and six patients undergoing elective coronary artery bypass grafting (CABG) received either levosimendan (24µg/kg i.v. bolus) or a placebo before CPB. This resulted in a reduction of tracheal intubation time (11.3 hours in the levosimendan group vs. 13.6 hours in the placebo group, p=0.02), decreased requirement for inotropic support for more than 12 hours (2% in the levosimendan

group vs. 9% in the placebo group, $p=0.02$) and shortened stay in the intensive care unit (ICU) (24.8 hours in the levosimendan group vs. 32.7 hours in the placebo group, $p=0.002$) (Tritapepe et al., 2009). A decrease in postoperative atrial fibrillation (FA) in CABG patients with LVEF $<30\%$ was seen when levosimendan was administered after induction of anaesthesia (incidence of FA 5%, $p<0.01$) compared to milrinone (incidence of FA 50%) or when levosimendan was started immediately after the release of the aortic cross-clamp (incidence of FA 35%) (De Hert et al., 2008). In patients with a preoperative LVEF $<30\%$ scheduled for elective CABG, the intraoperative combination of dobutamine and levosimendan was associated with a shorter tracheal intubation time, a shorter duration of inotropic drug administration and better maintained stroke volume (SV) compared to combined milrinone and levosimendan (De Hert et al., 2007).

The effect of starting the levosimendan infusion at different time points has been investigated by Tasouli et al. In their study patients were randomized to receive a continuous infusion of levosimendan ($0.1\mu\text{g}/\text{kg}/\text{min}$) either started intra- or postoperatively in the ICU on patients undergoing open-heart surgery. Both treatment strategies were safe and efficacious in increasing CI, mixed venous oxygen saturation (SvO_2) and LVEF. The earlier start was associated with shorter length of ICU stay (5 vs. 8 days, $p=0.002$) and hospital stay (9 vs. 15 days, $p=0.001$) (Tasouli et al., 2007).

Furthermore, a recent meta-analysis suggested that the use of levosimendan in patients undergoing cardiac surgery was associated with a significant reduction in postoperative cardiac troponin I and length of hospital stay (Zangrillo et al., 2009). The authors hypothesized that better preservation of early cardiac function with levosimendan may have been a result of the improved global tissue perfusion leading to better recovery from surgery. Another meta-analysis including 440 patients for 10 randomized controlled trials found an association between levosimendan and a significant reduction in postoperative mortality (11/235 [4.7%] in the levosimendan group vs. 26/205 [12.7%] in the control group), in which the patients were treated with placebo, dobutamine or milrinone (Landoni et al., 2010). In view of the current literature, levosimendan should be considered in with high-risk cardiac surgery patients. The cardioprotective ability of levosimendan and reduction in mortality has been documented in recently published meta-analyses. The vasodilating effect of levosimendan may lead to hypotension, which may be a

problem in the vasodilatory conditions such as CPB and anaesthesia. A vasopressor is usually needed to control the hypotension in surgical patients. The positive results of shorter ICU and hospital stay should be verified by larger, randomized trials.

2.3.2. Cardiogenic shock

Cardiogenic shock (CS) is the leading cause of death in patients hospitalized for acute myocardial infarction with mortality rates up to 60% (Goldberg et al., 1999). It is defined as the inability of the heart, as a result of impairment of its pumping function, to deliver sufficient blood flow to the tissues to meet metabolic demands (Califf and Bengtson, 1994). The diagnosis is a combination of low arterial blood pressure (SAP<90mmHg), low CI (<2.2l/min/m²), elevated pulmonary capillary wedge pressure (PCWP>16mmHg), low urine output (< 0.5ml/kg/h), elevated HR (>90) and signs of hypoperfusion (Nieminen et al., 2005). The rapid stabilization and treatment of reversible causes (for example revascularization) is the cornerstone of care. Mechanical assist devices, inotropes and vasopressor agents are used in patients with CS (Nieminen et al., 2005) causing personnel burden, high costs and prolonged hospital stay.

There are two main theories behind the use of levosimendan in CS. First, the use of standard inotropes is potentially harmful as, unlike levosimendan, they increase oxygen demand and intracellular calcium loading (Nieminen et al., 2005). Secondly, systemic inflammation and secondary multiple organ dysfunction / failure are the leading causes of death in patients with CS (Buerke and Prondzinsky, 2008). Levosimendan reportedly has positive effects on microcirculation (Schwarte et al., 2005) and have anti-inflammatory effects (Boost et al., 2008).

In a small, observational study of 10 patients, patients with CS received levosimendan as a continuous infusion (0.1µg/kg/min for 24 hours) as add-on therapy to fluids and catecholamines. The main effect observed was an increase in CI and a decrease in systemic vascular resistance (SVR) (Delle Karth et al., 2003). In a larger observational study 56 patients with persistent CS for 24 hours after revascularization were additionally treated with levosimendan (a bolus of 12µg/kg for 10 minutes + infusion 0.05-0.02µg/kg/min for 24 hours). Thirty-one patients

received the standard treatment and 25 patients the levosimendan treatment. Levosimendan produced a significant increase in CI and cardiac power index whereas SVR decreased significantly. There was no significant change in blood pressure during levosimendan treatment (Russ et al., 2007). A prospective, randomized, controlled single-centre trial compared the effects of levosimendan (a bolus of 12µg/kg for 10 minutes + infusion 0.1µg/kg/min for 50 minutes and infusion 0.2µg/kg/min for the next 23 hours) with a PDE- III inhibitor enoximone. Thirty-two patients with refractory CS for at least 2 hours requiring additional therapy were included in the study. A key finding of the study was a significant reduction in all-cause mortality rate at 30 days in the levosimendan treated patients. Furthermore, multiple organ failure leading to death (n= 4/10) occurred only in the enoximone group (Fuhrmann et al., 2008). On the other hand, in an observational study with 46 patients treated with levosimendan and 48 patients with standard therapy suffering from CS due to ST elevation myocardial infarction there was no difference in mortality between the groups (Omerovic et al., 2010).

There is no large randomized trial proving the benefit of levosimendan treatment in the CS. Although there has been encouraging results from small clinical trials levosimendan is still not recommended as therapy for CS.

2.3.3. Sepsis

Sepsis is the manifestation of immune and inflammatory response to infection (Ayres, 1985). A microbiologically proven or suspected infection together with systemic inflammatory response syndrome is defined as sepsis. Severe sepsis is related to acute organ dysfunction, hypoperfusion or sepsis-induced hypotension. Septic shock is defined as severe sepsis with hypotension that persists despite adequate fluid resuscitation or with signs of hypoperfusion. The definitions recognize the importance of myocardial depression and include cardiac dysfunction as one of the criteria for diagnosis of severe sepsis (Bone et al., 1992).

Sepsis is a leading cause of death in critically ill patients (Angus et al., 2001). In severe sepsis left ventricular dysfunction has been reported to be present in 24-72% of cases (Angus et al., 2001; Hoste et al., 2003; Vincent et al., 2006; van Gestel et al., 2004). Fluid resuscitation alone can alleviate haemodynamic disturbances only

in a mild cardiovascular dysfunction and patients with septic shock need vasoactive support in 85% of cases (Annane et al., 2003).

Clinically, sepsis has been characterized by a hyperdynamic state, with tachycardia, normal-to-low blood pressure, normal-to-high CI, and a low SVR (Hollenberg et al., 2004). Despite the increase in CO during the hyperdynamic phase of sepsis, studies indicate myocardial dysfunction. Increased HR and regional vascular alterations are the main variables that may impair myocardial performance. Both right and left ventricles may dilate, contractile function may decrease and ventricular compliance is reduced (Kumar et al., 2000). Severe reduction in ejection fraction has been reported in septic patients despite normal or elevated CI (Parker et al., 1984). The myocardial adrenergic hyporesponsiveness may persist for several days, resolving within 8-10 days (Parker et al., 1984; Cariou et al., 2008). Despite correction of global haemodynamic alterations, many patients may still suffer from regional and microvascular blood flow decrease, which may lead to multiple organ failure (Poeze et al., 2005; Sakr et al., 2004).

The effects of levosimendan in endotoxemia and sepsis have been studied in various experimental studies and a few clinical studies. The most significant experimental studies are summarized in Table 2. Theoretically, levosimendan may have beneficial effects in septic myocardial depression due to its mechanism of action. The improvements in the functions of both ventricles are independent of changes in intracellular Ca^{2+} - concentrations by increasing contractile myofilament sensitivity to Ca^{2+} - ions (Pinto et al., 2008). It also causes vasodilation by opening ATP-sensitive potassium channels which may result in improved tissue oxygenation (Yokoshiki et al., 1997b; Pinto et al., 2008). The experimental data also suggest that inhaled levosimendan reduces release of inflammatory mediator in ventilator-induced lung injury (Boost et al., 2008).

Reference	Subjects	Intervention groups	Effect of levosimendan
Oldner et al., 2001	14 endotoxemic pigs	Levosimendan (bolus 200µg/kg + infusion 200µg/kg/h) Control (all effects ^c)	Increase in CI, DO _{2i} , VO _{2i} Decrease in MPAP, PVRI Increase in DO _{2igut} , VO _{2igut} No change in arterial pH, lactate or renal artery blood flow
Zager et al., 2006	Endotoxemic mice	Multiple experiments	LPS-induced ARF: decrease in BUN and plasma creatinine No change in TNF-α or MCP-1 Prevention of ANG-II mediated MC contraction
Dubin et al., 2007	19 endotoxemic sheep	Levosimendan (100µg/kg + infusion 100 µg/kg/h) Dobutamine (10µg/kg/min) Control	Increase in CO ^c , SvO ₂ ^c Increase in superior mesenteric artery blood flow ^{c,d} , intestine DO ₂ ^{c,d} , mesenteric SvO ₂ ^{c,d} Decrease in ΔPCO ₂ No change in arterial lactate
Cunha-Goncalves et al., 2007	12 endotoxemic pigs	Levosimendan (infusion 50µg/kg/h) Control (all effects ^c)	Increase in HR No change in CO, SVR No change in DO ₂ , VO ₂ Decrease in SvO ₂ , coronary perfusion pressure
Fries et al., 2008	32 endotoxemic rats + 8 sham controls	Fluid resuscitation (25 ml/kg/h) (Fr) Fr + norepinephrine (0.5µg/kg/min) Fr + levosimendan (0.3µg/kg/min) No treatment Control	No change in CO ⁿ , microvascular blood flow ⁿ Increase in microvascular oxygenation ⁿ
Cunha-Goncalves et al., 2009a	16 endotoxemic pigs	Levosimendan (infusion 50µg/kg/h) Control (all effects ^c)	No change in CO or HR, decrease in MAP No change in hepatic artery, superior mesenteric artery, portal vein flows No change in DO ₂ , VO ₂
Cunha-Goncalves et al., 2009b	18 endotoxemic pigs	Levosimendan (infusion 25-50µg/kg/h) Dobutamine (10-20µg/kg/min) (all effects ^d) Control	No change in CO, HR, MAP Decrease in portal vein flow No change in hepatic artery and superior mesenteric artery flows Decrease in hepatic vein oxygen saturation
Rehberg et al., 2010	21 sheep	Levosimendan (0.2µg/kg/min) + arginine vasopressin (0.5mU/kg/min) Arginine vasopressin (0.5mU/kg/min) Control	Increased LSVWI ^{a,c} Decreased net fluid balance ^{a,c} Improved survival ^{a,c} Did not increase norepinephrine requirements Decreased PVRI ^{a,c}

Scheiermann et al., 2009	21 endotoxemic rats + 7 sham controls	Levosimendan (24µg/kg i.v.) Levosimendan (24µg/kg inh.) No treatment (all effects ^s) Control	i.v. and inh. levosimendan increased MAP, arterial pH, prolonged survival Decrease in proinflammatory cytokine levels
Garcia-Septien et al., 2010	14 endotoxemic pigs + 7 sham controls	Levosimendan (bolus 200µg/kg + infusion 200µg/kg/h) No treatment Control	Decrease in PVRI ^{c,s} Decrease in ΔPCO ₂ ^s Improved portal blood flow ^s No change in urine output ^s

Table 2. Experimental trials on levosimendan in endotoxemia. The superscripts indicate statistically significant difference of levosimendan from arginine vasopressin (^a), control (^c), dobutamine (^d), septic animal (^s), norepinephrine (ⁿ). ANG-II, angiotensin II; ARF, acute renal failure; BUN, blood urea nitrogen; CO / I, cardiac output / index; DO₂i, systemic oxygen delivery index; HR, heart rate; inh., inhaled; LPS, lipopolysaccharide; LVSWI, left ventricle stroke work index; MAP, mean arterial pressure; MC, mesangial cell ; MPAP, mean pulmonary arterial pressure, MCP-1, monocyte chemoattractant protein 1; ΔPCO₂, intramucosal-arterial partial pressure of carbon dioxide; PVRI, pulmonary vascular resistance index; SV, stroke volume; SVR, systemic vascular resistance; SvO₂, mixed venous oxygen saturation; TNF-α, tumour necrosis factor alpha; VO₂ / i, systemic oxygen consumption / index .

A case report in 2005 described the first successful use of levosimendan in an immunocompromised patient with septic shock (Matejovic et al., 2005). Since then, a case report of two patients (Noto et al., 2005) and a case series of six patients (Powell and De Keulenaer, 2007) have stated the beneficial effects of levosimendan.

Morelli et al. published the first randomized, controlled clinical trial evaluating the effects of levosimendan in patients suffering from septic shock (Morelli et al., 2005). The inclusion criterion was persistent LVEF below 45% after 2 days of conventional treatment (volume substitution, norepinephrine and dobutamine). Twenty-eight patients were randomized to receive either continuous infusion of 0.2µg/kg/min levosimendan over 24 hours or 0.5µg/kg/min dobutamine. The levosimendan improved the left ventricle stroke work index, end-systolic and end-diastolic volume index, increased oxygen consumption and delivery index and urinary output, and reduced arterial lactate levels and gastric mucosal hypoxia compared to dobutamine. The groups did not differ in norepinephrine infusion rate.

The same group investigated the effects of levosimendan on septic shock patients with acute respiratory distress syndrome and right ventricular failure (Morelli et al., 2006). Thirty-five patients were randomized to receive either continuous infusion of 0.2µg/kg/min levosimendan or placebo. Levosimendan reduced PVRI and increased right ventricular function measured by the increase in the right ventricular ejection fraction and decrease in the right ventricular end-systolic volume index. Levosimendan also significantly increased SvO₂ and oxygen delivery, which was not seen with placebo.

Summarizing the most recent literature, levosimendan may become a promising alternative in patients suffering from severe sepsis or septic shock. However, there are also some controversial results in the experimental studies of the beneficial effects of levosimendan especially in splanchnic perfusion. Because of the vasodilating properties of levosimendan, continuous haemodynamic monitoring should be used and the optimal volume status of the patient in combination of vasopressor agent ensured to prevent side effects.

2.3.4. Noncardiac surgery

HF carries a significant risk of perioperative morbidity and mortality. Mortality in HF patients is twofold higher after major noncardiac surgery than in patients with coronary artery disease or general population (Hernandez et al., 2004).

No randomized, controlled trials have been reported on patients with preoperative low output syndrome treated with levosimendan. However, the first results from small clinical trials in this patient category with prophylactic levosimendan have been published.

The effects of prophylactic levosimendan administration were studied in 12 HF patients with poor left ventricular function (preoperative LVEF < 30%) undergoing abdominal surgery. Levosimendan was administered one day before surgery in the ICU under continuous haemodynamic monitoring. Levosimendan resulted in a significant increase in LVEF from baseline value to day 7. No adverse reactions, complications or mortality occurred in the 30-day follow up period (Katsaragakis et al., 2008). In another study, 10 patients with symptomatic HF and poor left ventricular function (LVEF < 35%) received preoperative levosimendan infusion two hours before emergency hip fracture repair. Levosimendan increased CI and SI from baseline to 48-hour follow-up (Ponschab et al., 2008).

2.3.5. Other critical care studies

2.3.5.1. Intoxication

Levosimendan has been studied in two experimental settings in the treatment of local anaesthetic-induced cardiodepression. In a study with an isolated guinea pig heart, levosimendan was capable of reversing the ropivacaine-induced myocardial contractile depression but the myocardial sensitivity and efficacy for levosimendan did not change (Stehr et al., 2007). The systemic effect of levosimendan on severe myocardial depression caused by local anaesthetic has also been studied by Aittomäki et al. (Aittomäki et al., 2010). In their experimental study twenty pigs received bupivacaine 2mg/kg/min until MAP decreased 55% from baseline.

Levosimendan (80µg/kg for 10 minutes followed by 0.7µg/kg/min for the next 50 minutes) together with fluid resuscitation reversed the cardiac depression, but there was no difference in overall recovery in comparison to treatment with fluids only.

There is a case report showing the life-saving effect of levosimendan on stunned myocardium induced by carbon monoxide poisoning (carboxyhemoglobin 43%). A 32-year-old woman suffered severe cardiac failure shown by pulmonary artery catheter and cardiac magnetic resonance despite dobutamine infusion. Levosimendan improved LVEF from 18.4% to 46.8% and CI from 1.8l/min/m² to 3.4l/min/m² in 24 hours (Rocco et al., 2006).

The use of levosimendan in calcium channel blocker overdose has been described in three experimental studies not favouring and two case reports favouring the use of levosimendan. In the experimental studies, levosimendan in different dosages was compared to calcium chloride (CaCl₂) and potassium channel antagonist 4-aminopyrine (4-AP). In the case reports, standard treatment was inefficacious and levosimendan was added as a rescue medication. The studies and the most important results are summarized in Table 3.

Reference	Subjects	Intervention groups	Effect of levosimendan	Recommendation
Graudins et al., 2008	35 rats	1. Control 2. CaCl ₂ 3. Levosimendan (6µg/kg over 10 min + infusion 0.4µg/kg/min for 60 min) 4. Levosimendan (24µg/kg over 10 min + infusion 0.6µg/kg/min for 60 min) 5. Levosimendan (0.4µg/kg/min for 70 min) + CaCl ₂	Increase in CO No improvement in BP No further improvement in haemodynamics than CaCl ₂ No effect in survival	Not recommended
Abraham et al., 2009	14 rats	Control Levosimendan (12µg/kg + 18µg/kg after 5 min)	Decrease in survival	Not recommended
Graudins and Wong, 2010	60 rats	1. Control 2. Levosimendan (6.25µg/kg over 10 min + infusion 36µg/kg/h) 3. 4-AP 4. Levosimendan (6.25µg/kg+ infusion 36µg/kg/h) + 4-AP 5. CaCl ₂ 6. Levosimendan (6.25µg/kg+ infusion 36 µg/kg/h) + CaCl ₂	4-AP more effective than levosimendan improving haemodynamics Levosimendan + 4-AP: no additive increase in CO, BP	Not recommended
Varpula et al., 2009	1 female 1 male	Case report Standard treatment + levosimendan	Improvement in haemodynamics	Recommended
Osthoff et al., 2010	1 male	Case report Standard treatment + levosimendan	Improvement in haemodynamics	Recommended

Table 3. Levosimendan in calcium channel poisoning. CaCl₂, calcium chloride; 4-AP, 4-aminopyridine; CO, cardiac output; BP, blood pressure.

2.3.5.2. Effect in splanchnic blood flow

A decrease in splanchnic perfusion is a risk factor for postoperative complications (Mythen and Webb, 1994) and also for multiple organ failure (Swank and Deitch, 1996). The reported incidence of gastrointestinal complications after cardiac operations is relatively low (0.8-3.7%), but the associated mortality is high (13.9-86.9%) (Musleh et al., 2003). In patients undergoing CPB of more than 80 minutes, splanchnic perfusion and hepatocellular integrity were moderately affected (Kumle et al., 2003). Inadequate visceral perfusion may lead to a breakdown of the intestinal mucosal barrier (Swank and Deitch, 1996) leading to a translocation of endotoxines and microorganisms (Riddington et al., 1996).

Major vascular surgery can also be associated with an imbalance between splanchnic oxygen supply and demand. Cornet et al. have shown that hepatosplanchnic ischaemia/reperfusion is a major risk factor for pulmonary vascular injury associated with aortic surgery (Cornet et al., 2009). The major defence mechanism protecting mucosal integrity is adequate microcirculatory perfusion and oxygenation (Sato et al., 1988). Therefore, prophylactic and therapeutic interventions are required to increase splanchnic perfusion and oxygenation.

Levosimendan may, by its pharmacologic mechanism, increase CO and simultaneously redistribute perfusion towards splanchnic organs due to its vasodilatory properties. Experimental data indicate that levosimendan is superior to milrinone and dobutamine in selectively increasing microvascular gastric mucosal oxygenation (Schwarte et al., 2005). It also increased blood flow to the small intestine and liver and reduced vascular resistance in these organs in an experimental setting in dogs (Pagel et al., 1996). The portal venous blood flow and oxygen delivery were increased in experimental septic shock (Oldner et al., 2001). The better regional tissue perfusion and vasodilation was seen in parallel with improvement in cardiac performance.

On the other hand, undesired systemic side effects of levosimendan, such as hypotension and tachycardia, may evoke splanchnic vasoconstriction thereby decreasing mucosal oxygenation. The strong vasodilatory properties of the drug were probably involved in the negative response seen in two experimental studies evaluating the impact of levosimendan on global hemodynamics and splanchnic

circulation in a pig model of early septic shock induced by endotoxin injection (Cunha-Goncalves et al., 2009a; Cunha-Goncalves et al., 2009b). These studies suggest that levosimendan is not a splanchnic vasodilatory drug, and that the beneficial effect of levosimendan reported in some studies is probably related to changes in CO rather than to specific splanchnic effects. Most importantly, levosimendan should not be given before correction of hypovolemia.

2.3.5.3. Cardiopulmonary resuscitation

The experience with levosimendan during cardiopulmonary resuscitation (CPR) is restricted to experimental studies and case reports. Koudouna and co-workers induced ventricular fibrillation (VF) in 20 piglets and left them untreated for 8 minutes. The animals were randomized to receive levosimendan (0.012mg/kg) or placebo combined with epinephrine (0.02mg/kg) at the beginning of CPR. The levosimendan group had significantly better initial resuscitation success (4/10 in the placebo group vs. 10/10 in the levosimendan group) and coronary perfusion pressure improved during CPR in the levosimendan group (Koudouna et al., 2007). In a preliminary study of prolonged cardiac arrest in rat model, dobutamine and levosimendan had comparable haemodynamic effects. However, levosimendan offered greater survival benefit in association with smaller increases in HR and more favourable filling pressures (Huang et al., 2005a). In a porcine model, resuscitated animals were randomized to treatment with high dose of levosimendan (bolus 20 μ g/kg over 10 min and infusion 0.4 μ g/kg/min for 220 min), dobutamine (5 μ g/kg/min for 230 min) or placebo. Levosimendan and dobutamine produced a comparable increase in CO. However, contractile function measured by LVEF and fractional area change was significantly better in the levosimendan group than in the dobutamine and placebo groups (Huang et al., 2005b). The beneficial effects of levosimendan during CPR may not only be due to the positive inotropic effects of the compound. In a rat model of VF, the positive effects of levosimendan (fewer shocks, lower ST-segment elevations and improved survival) were abolished by prophylactic administration of the non-selective K⁺ channel inhibitor, glibenclamide (Cammarata et al., 2006). The capability to minimize myocardial ischaemic injury

during cardiac arrest may be explained by levosimendan's K_{ATP} - channel agonist effects.

There are two case reports suggesting the beneficial effects of levosimendan during CPR. A 32-year-old man with severe congestive HF due to idiopathic cardiomyopathy (LVEF of 32%) developed ventricular tachycardia followed by electromechanical dissociation. Haemodynamics could not be restored with conventional CPR with high doses of inotropic medications. Levosimendan infusion $0.3\mu\text{g}/\text{kg}/\text{min}$ was added to the resuscitation procedure and haemodynamics was restored after 2.5 h of CPR. At a control visit 17 months later, his neurological state was normal and clinical condition stable (Tsagalou and Nanas, 2006). A 39-year-old woman suffering from post-partum atonic uterine bleeding had a catecholamine-resistant cardiac arrest and fulminant pulmonary failure due to the side-effects of treatment with prostaglandines. The return of spontaneous circulation was achieved after more than 90 minutes of CPR and at that point echocardiography revealed severe right and left ventricular contractile dysfunction (LVEF < 10%). After adding levosimendan ($12\mu\text{g}/\text{kg}$ bolus followed by a $0.2\mu\text{g}/\text{kg}/\text{min}$ continuous infusion), circulation stabilized within 30 minutes and LVEF was 45% (Krumnikl et al., 2006).

A study investigating and comparing the inotropic effects of levosimendan, dobutamine and milrinone at various temperatures suggested that levosimendan produces targeted inotropy under hypothermia. By contrast, the inotropic effect of dobutamine and milrinone was suppressed at 31°C and 34°C . This might be clinically significant during neuroprotective hypothermia following successful CPR (Rieg et al., 2009).

2.3.5.4. Pulmonary hypertension

Levosimendan was compared with placebo in 21 patients with chronic pulmonary hypertension (mPAP > 30mmHg). Levosimendan was administered with a loading dose of $12\mu\text{g}/\text{kg}$ and an infusion of $0.1\text{-}0.2\mu\text{g}/\text{kg}/\text{min}$ for 24 hours. The study drug was readministered 4 times at 2-week intervals as an infusion of $0.2\mu\text{g}/\text{kg}/\text{min}$ for 6 hours. The initial 24-hour infusion produced a significant reduction in PVR, mPAP

and SvO₂, but the difference between the groups disappeared at 8 weeks. The authors postulate that a higher dose or a shorter dosing interval might have been more effective (Kleber et al., 2009). In decompensated HF patients, levosimendan led to a reduction in PVR (Slawsky et al., 2000) and significant benefit in right ventricle efficiency (Ukkonen et al., 2000). Improvements in the right ventricle function and reduction in PVR have also been seen in ischaemic right ventricle failure (Russ et al., 2009; Parissis et al., 2006), ARDS (Morelli et al., 2006) and in two case reports after mitral valve replacement surgery (Cicekcioglu et al., 2008; Morais, 2006). However, in a report of two cases, levosimendan was found to increase pulmonary pressures in patients with nonvasoreactive idiopathic pulmonary artery hypertension (Cavusoglu et al., 2009).

2.3.5.5. Viral myocarditis

Acute viral myocarditis is an inflammatory condition that leads to acute HF through the excessive loss of cardiomyocytes in the absence of an ischaemic event (Esfandiarei and McManus, 2008). Apoptosis is involved in the pathogenesis of HF and myocarditis (Kytö et al., 2004). Levosimendan has been found to inhibit cardiomyocyte apoptosis in an animal model of hypertension-induced heart disease (Louhelainen et al., 2007). In experimental acute HF caused by coxsackie virus B3 myocarditis, levosimendan treatment decreased the amount of apoptotic cardiomyocytes by 40% in severe myocarditis and significantly improved left ventricular function (Latva-Hirvelä et al., 2009). Clinical studies using levosimendan in viral myocarditis are lacking. A case report on two patients suffering from infectious myocarditis caused by acute Chagas' disease (caused by protozoan *Trypanosoma cruzi*) describe the improvements in cardiac performance with administration of levosimendan (de March Ronsoni et al., 2009).

2.3.5.6. *Obstetrics*

Peripartum cardiomyopathy (PPCM) is a rare cause of acute HF in the last months of pregnancy or within 5 months of delivery. The diagnostic criteria include absence of preexisting cardiac disease and identifiable cause of cardiac failure with echocardiographic evidence of left ventricular systolic dysfunction. The treatment of PPCM is similar to that of other forms of HF aiming to reduce afterload and preload and to increase contractility (Moioli et al., 2010). The knowledge of using levosimendan in this entity is limited because of its rarity and is based on case series and retrospective data. A case report in 2004 described the first-time use of levosimendan in PPCM. Levosimendan induced a steady decline in PCWP, improved SV and increased LVEF from below 20% to 45% at day 9 and further over 60% after week 10 (Benlolo et al., 2004). Since then, three more case reports with favourable results have been published (Nguyen and McKeown, 2005; Benezet-Mazuecos and de la Hera, 2008; Uriarte-Rodriguez et al., 2010).

Levosimendan exerted a potent relaxant effect on spontaneous and agonist-induced human uterine myometrium cell cultures. The authors discuss the possible potential of levosimendan for clinical use for preterm labour (Hehir et al., 2010).

2.3.5.7. *Brain injury*

A preliminary report on experimental brain injury investigated the ability of levosimendan to prevent the vasospasm after subarachnoid haemorrhage in a rabbit model. Experimental data suggest that calcium dependent and independent vasoconstriction takes place during vasospasm among at least free radical reactions, an imbalance between vasoconstrictor and vasodilator substances, inflammatory processes, apoptosis and a disorder of the neuronal mechanisms that regulate vascular tonus. The preischemic levosimendan infusion before cerebral vasospasm protected against apoptosis, increased the pathological luminal area of the basillary artery and reduced muscular wall thickness measurements (Cengiz et al., 2010).

A case report of a 38-year-old woman with no previous cardiac disease history described a cardiogenic shock with subarachnoid haemorrhage. The cerebral perfusion pressure could not be optimized by conventional haemodynamic management. Levosimendan was added to the therapy to counteract the myocardial stunning. There was a rapid improvement in LVEF, left ventricle wall motion and filling pressures. The intracranial pressure was not measured (Busani et al., 2010).

2.3.5.8. Effect in kidney function

Beneficial effects of levosimendan have been also found for kidney functions. The effect of levosimendan administration on renal function was compared to standard treatment in advanced chronic HF patients awaiting heart transplant. Some improvement in kidney function was found in levosimendan treated patients (Zemljic et al., 2007). In septic patients treated with levosimendan the creatinine clearance increased 64% compared to no improvement in the dobutamine group and the effect of levosimendan on renal function was confirmed by the increase in urine output (Morelli et al., 2005). In the LIDO study, there was a significant decrease in serum creatinine in the levosimendan group compared to the dobutamine group (Follath et al., 2002). The beneficial renal effect of levosimendan may be an extension of higher CO. However, an experimental study by Zager et al. suggests an independent additional value for kidneys, possibly by vasodilation (Zager et al., 2006).

3. Aims of the study

The aim of this study was to assess the effects of levosimendan in new clinical and experimental scenarios. The specific objectives were:

- 1) To assess the effects of intraoperative levosimendan on systemic and splanchnic circulation during and after abdominal aortic surgery. (I)
- 2) To assess the effects of preoperative levosimendan on haemodynamics, formation of metabolites OR-1855 and OR-1896 and splanchnic circulation in patients undergoing aortic valve replacement (AVR) together with CABG. (II)
- 3) To assess in an experimental model the effects of levosimendan during severe calcium antagonist intoxication. (III)
- 4) To assess whether levosimendan could reverse propranolol-induced severe negative inotropy in a porcine model of β -blocker intoxication. (IV)

4. Subjects and methods

The studies can be summarized as follows:

Study	Randomization	Double-blind	Groups	N	Setting	Main objective
I	yes	yes	levosimendan placebo	10 10	intraoperative levosimendan in aortic aneurysm surgery patients	splanchnic perfusion
II	yes	yes	levosimendan placebo	12 12	preoperative levosimendan in CABG + AVR patients	haemodynamics, formation of OR-1896 and OR-1855.
III	yes	no	levosimendan placebo	6 6	verapamil intoxication in pigs	survival and haemodynamic performance
IV	yes	yes	levosimendan dobutamine placebo	9 9 6	propranolol intoxication in pigs	haemodynamic performance

4.1. Study subjects

Studies I and II had specific inclusion and exclusion criteria which the subjects had to fulfil before being included in the study. The inclusion criteria were

Study I: patients with an infrarenal abdominal aortic aneurysm undergoing elective surgery were included in the study. The main exclusion criterion was aortic valvular disease.

Study II: patients undergoing combined AVR and CABG with LVEF less than 50% or left ventricular hypertrophy indicated by wall thickness more than 12mm were included in the study. The exclusion criterion was allergy to levosimendan.

Study III and IV: 36 young pigs of either sex were included. All the animals were brought to the National Animal Laboratory Centre 7 days before the experiment to avoid stress reactions. They were from the same farm, of the same age and the same weight (mean 29kg, SD=2) with no difference between the groups. The pigs were deprived of food but not water 12 hours before the experiments.

4.2. Methods

4.2.1. Study designs

Study I was designed to find out if levosimendan could improve splanchnic blood flow and tissue perfusion in major vascular surgery. The patients received a levosimendan (24µg/kg) or placebo bolus over 30 minutes before induction of anaesthesia. The levosimendan (0.2µg/kg/min) or placebo infusion was continued for 24 hours.

Study II was designed to ascertain the effects of preoperatively infused levosimendan (12µg/kg bolus followed by an infusion of 0.2µg/kg) on haemodynamics in combined AVR and CABG. Anaesthesia, surgery and postoperative treatment were performed according to the local standards.

Study III assessed the effects of levosimendan in severe verapamil poisoning. The study design is described in Figure 5.

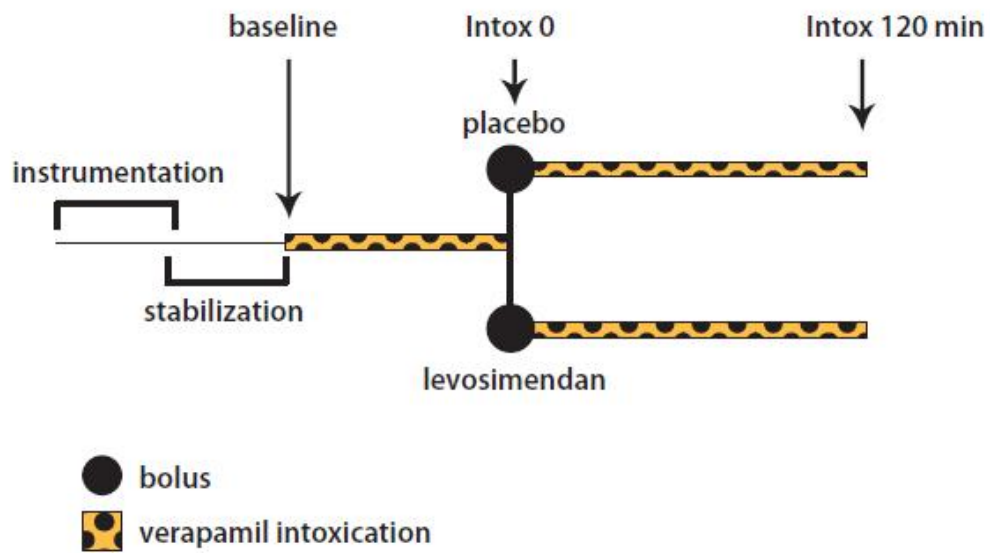


Figure 5. Design of Study III. There were six pigs in the levosimendan and placebo groups.

Study IV was intended to assess the effect of levosimendan on haemodynamic performance in a porcine model of propranolol-induced myocardial depression. The design of the study is described in Figure 6.

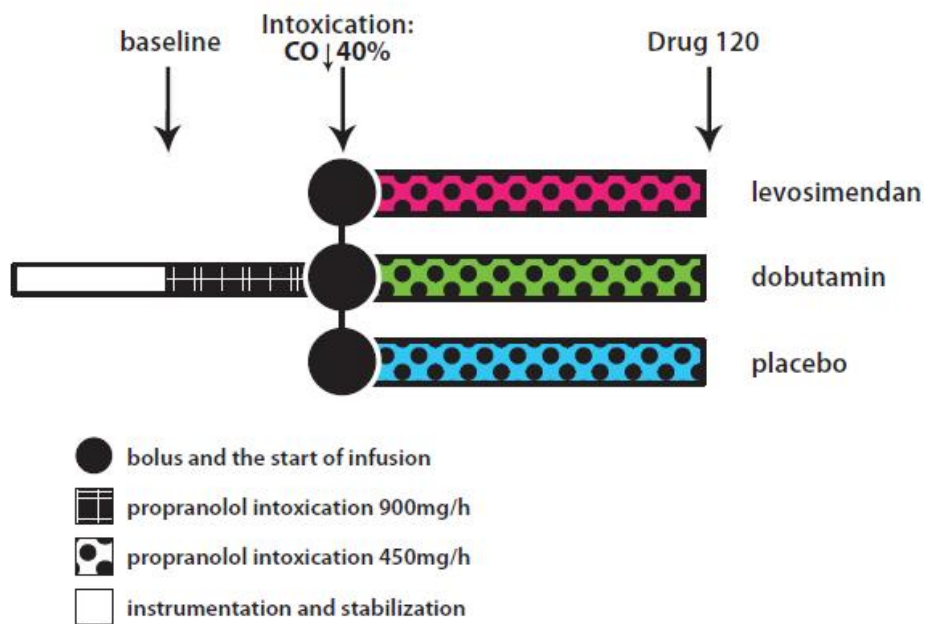


Figure 6. Design of Study IV. There were nine pigs in the levosimendan and dobutamine groups and six pigs in the placebo group.

4.2.2. Haemodynamic measurements and monitoring

Study I: A radial arterial cannula (BP) and a pulmonary artery catheter (CO, central venous pressure (CVP) and PCWP) were inserted under local anaesthesia. CO was measured by bolus injectates in triplicates. Standard 12-lead ECG recordings were obtained before surgery, immediately postoperatively and on the first postoperative day.

Study II: A radial arterial cannula was inserted for blood pressure measurements. The pulmonary artery catheter was used to measure CO, CVP and PCWP. Our standard measurements, LVEF, left ventricular mass, cardiac dimensions, mitral and aortic flow patterns and mitral annular velocities were recorded by echocardiography. Whole-body impedance cardiography (ICG_{WB}) was used to measure SI, HR, CI, left cardiac work index, systemic vascular resistance index (SVRI) and extracellular water preoperatively and on the first and fourth postoperative day.

Studies III and IV: The haemodynamic measurements were done by arterial cannulation (blood pressure), pulmonary artery catheter (CO, CVP and PCWP) and angiography catheter in the left ventricle (a change in the maximum of the positive slope of the left ventricular pressure). Heart rate was measured from ECG.

4.2.3. Visceral perfusion measurements

Study I: Systemic ICG-PDR was used to estimate the total splanchnic blood flow (LiMon[®], Pulsion Medical Systems, Germany). To assess a surrogate for gastric mucosal perfusion, an automatic gas tonometry (Tonocap[®], Datex Ohmeda, Finland) was used to measure the gastric mucosal partial pressure of carbon dioxide (ΔPCO_2).

Study II: The splanchnic perfusion was measured by vena hepatica catheter. The placement of the vena hepatica catheter was confirmed by fluoroscopy in the

operating theatre and by x-ray postoperatively. OER, an index of hepatosplanchnic metabolic function, was calculated according to the following equation: $OER (\%) = (CaO_2 - ChO_2) / CaO_2 * 100$, where CaO_2 is the arterial oxygen content and ChO_2 the hepatic venous oxygen content.

4.2.4. Laboratory assays

Study I: SvO_2 , $ScvO_2$, haemoglobin and lactate concentrations were measured before induction of anaesthesia, before and during aortic occlusion and at the end of surgery. Troponine T and P-creatinine kinase-MBm (P-CK-MBm) concentrations were measured on admission to the ICU and on the first postoperative morning. Creatinine was measured preoperatively and on the first and fourth postoperative days.

Study II: Blood gases and mixed venous saturation, lactate and haematocrite were taken before and after induction of anaesthesia, after weaning from perfusion, after sternum closure, on admission to the ICU and hourly thereafter for 24 hours. Levosimendan, OR-1896 and OR-1855 concentrations were measured at baseline, before induction of anaesthesia and at 72 and 96 hours. NT-proBNP was taken at baseline, before induction of anaesthesia and on the fourth postoperative morning. P-creatinine kinase and P-creatinine kinase-MB (P-CK-MB) subunit were taken 6 hours postoperatively and on the first and second postoperative morning. Blood samples from vena hepatica were collected after induction of anaesthesia, before unclamping of the aorta, after weaning from CPB, on arrival at the ICU and every 4 hours postoperatively until the first postoperative morning. Creatinine was measured preoperatively and on the first and fourth postoperative days.

Studies III and IV: Arterial and mixed venous blood-gas tensions and oxygen saturations, haemoglobin, lactate concentrations, sodium, potassium and glucose were measured.

4.2.5. Mortality

Studies I and II: Short-term mortality included ICU and hospital mortality. Long term mortality was determined as 6-month mortality. The data were obtained from the hospital records and Statistics Finland in November 2010.

Studies III and IV: Mortality was assessed during the 120-minute follow-up time.

4.3. Statistical analysis

For quantitative variables, results were summarized using descriptive statistics. All statistical calculations were performed using SPSS 14.0 (IV), 15.0 (I), 16.0 (III), 18.0 (II) software (SPSS INC, Chicago, IL). P-values of < 0.05 were considered statistically significant. Power analysis was performed in Studies I, II and IV.

Study I: The Friedman test was used for analysis of variance. When appropriate, the Wilcoxon signed rank test and Mann-Whitney U-test were used for further evaluation. The Chi-Square test was used for categorical variables. Values are presented as median and interquartile range.

Study II: The continuous variables were tested using Student's t-test and the categorical variables using Fisher's exact test. Changes in haemodynamic parameters were tested using repeated-measures analysis of variance (RANOVA). The Greenhouse-Geisser correction was used when the sphericity assumption, as assessed by Mauchly's test, was not met. Time point wise comparisons were done with the T-test. Cumulative doses of vasoactive medications were compared using Mann-Whitney U-test. Values are presented as mean \pm standard deviation.

Study III: Changes in haemodynamics and laboratory measures at preintoxication and postintoxication phases were evaluated by Mann-Whitney U-test. The Kaplan-Meier test was used for survival analysis. Values are presented as median and interquartile range.

Study IV: Mixed models were used to examine the differences between the study groups from preintoxication to to postintoxication phases. The effects of propranolol intoxication were presented as percentage change. Values are presented as median and interquartile range.

4.4. Ethical statement

Studies I and II were approved by the ethics committee of Pirkanmaa Hospital District and the National Agency for Medicines, Finland. Written informed consent was obtained from each patient in Studies I and II. The studies were performed in accordance with institutional guidelines and the Declaration of Helsinki. The National Animal Ethics Committee of Finland approved the study methodologies in Studies III and IV. Animal care, welfare and procedures were carried out in accordance with the regulations of the Council of Europe.

The studies were partly supported by the Competitive Research Funding of the Pirkanmaa Hospital District, Tampere University Hospital, the Finnish Cultural Foundation, the Ida Montin Foundation, Orion Pharma Ltd, the Orion-Farmos Research Foundation and the Finnish Medical Foundation.

5. Results

5.1. Effect on haemodynamics in surgical patients

In aortic surgery patients there was no difference in haemodynamic variables between the groups at baseline. During surgery until 60 minutes after aortic clamping the CI was higher in the levosimendan treated patients (Figure 7). SV remained comparable between the groups. Levosimendan was associated with stable HR while in the placebo group the HR decreased from baseline to 60 minutes after aortic clamping.

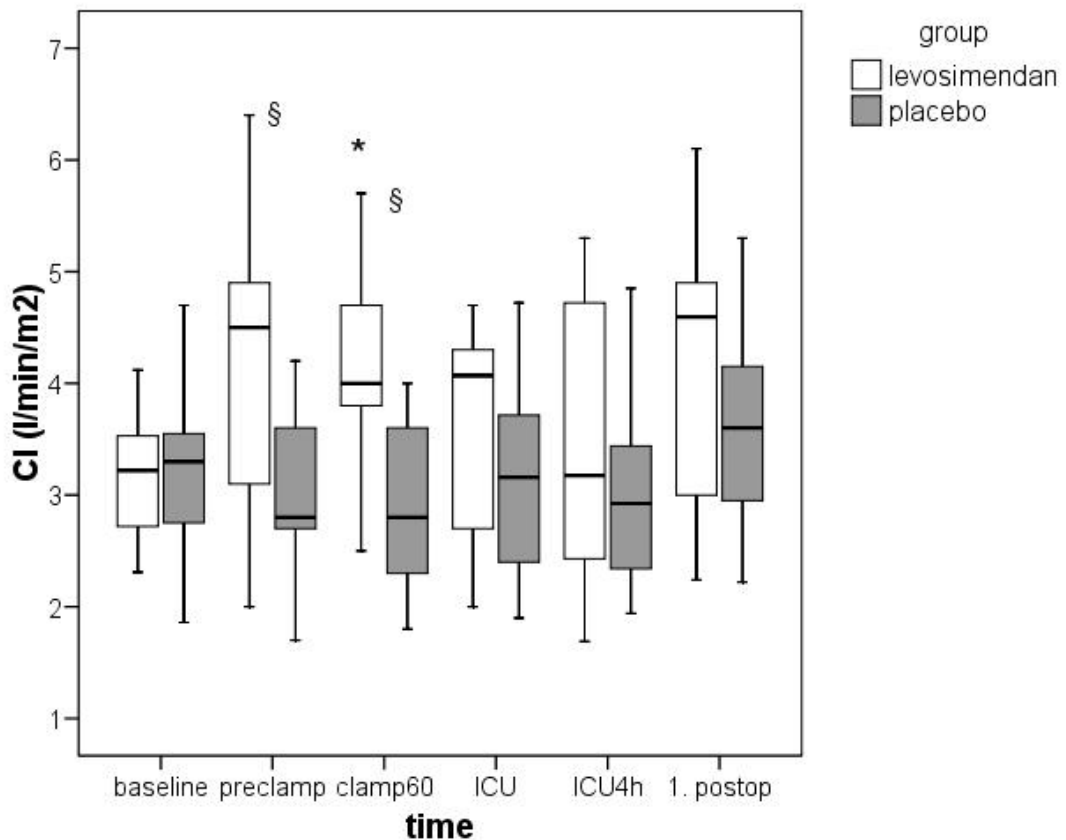
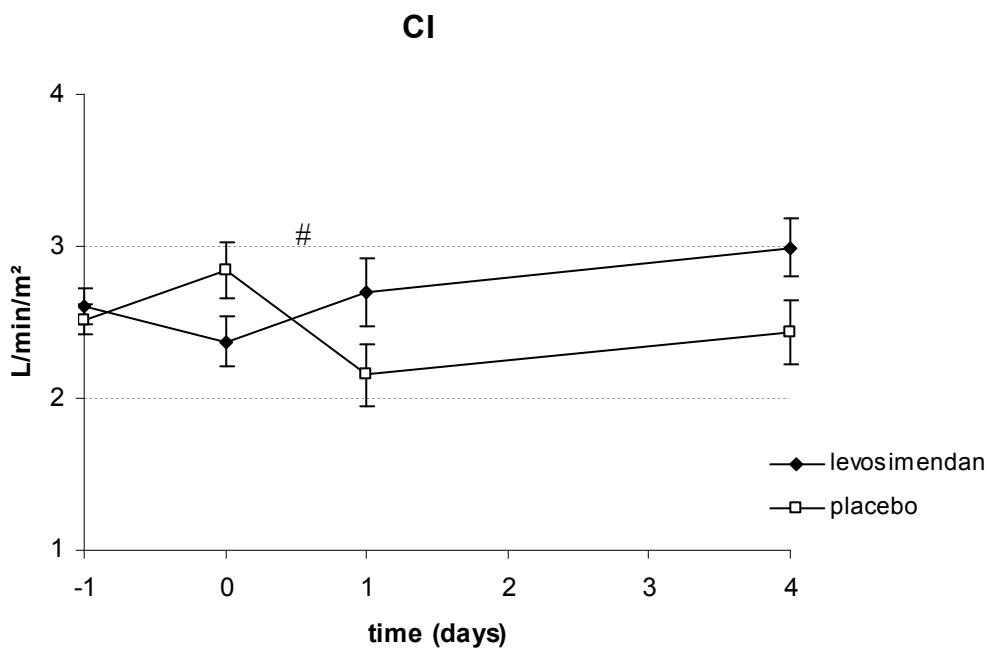


Figure 7. Cardiac index in the levosimendan (white columns) and placebo-treated patients (grey columns) at baseline, before aortic clamping (pre-clamp), 60 minutes

after aortic clamping (clamp60), on admission to the intensive care unit (ICU), 4 hours after admission (ICU4h) and on the first postoperative morning (1.postop). * $P < .05$ by Wilcoxon's signed-rank test (against baseline) and § $P < .05$ by Mann-Whitney U-test (between groups).

In the cardiac surgery patients, the CI and SI measured by ICG_{WB} are presented in Figure 8. There was no difference in HR or MAP between the groups. At baseline, transmitral flow velocity combined with annular velocity was elevated in both groups. Left atrial volume indices were also suggestive of diastolic dysfunction (39.4 ml/m² in the control group and 39.9 ml/m² in the levosimendan group). The LVEFs were normal in both groups at baseline. In the postoperative period, the LVEF dropped in the control group, but was maintained in the levosimendan group.



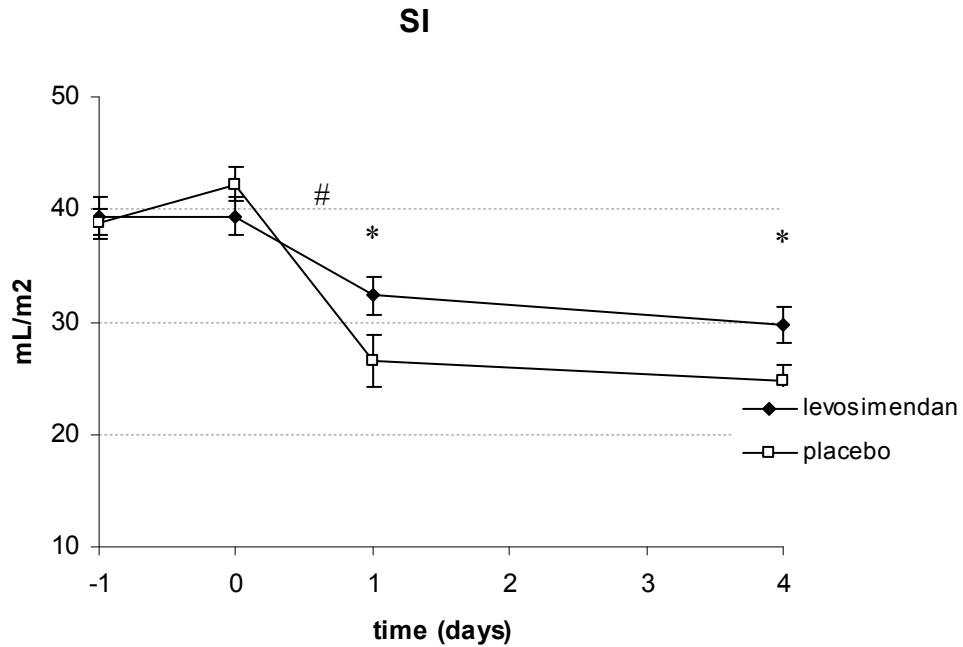


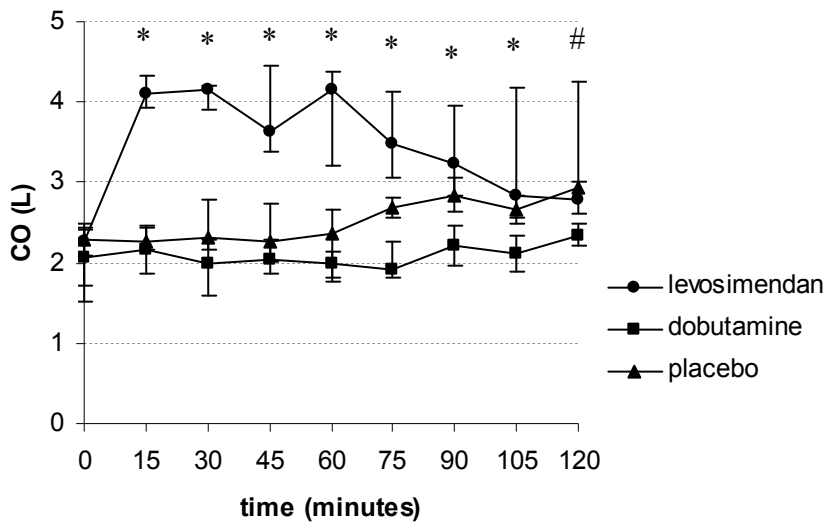
Figure 8. a) Cardiac index (CI) and b) stroke volume index (SI) at baseline (-1), on the morning of surgery (0), and on the first (1) and fourth (4) postoperative mornings measured by whole-body impedance cardiography. Results are expressed as mean \pm confidence intervals. * $P < 0.05$ between the groups and # the significant difference between the groups from the first postoperative morning to the morning before surgery.

In the aortic surgery study, six patients in the levosimendan group and three patients in the placebo group received norepinephrine during the operation ($P < 0.05$). Postoperatively four patients in the levosimendan group but none in the placebo group received norepinephrine ($P < 0.05$). In the cardiac surgery study, none of the patients needed norepinephrine during the study drug infusion. During the surgery and in the ICU, the levosimendan group received significantly more norepinephrine than the placebo group ($P < 0.05$).

5.2. Effect on haemodynamics in experimental animals

In the verapamil intoxication study, the baseline haemodynamics were similar between the levosimendan and placebo groups. After completion of intoxication (=time 0), the group receiving levosimendan showed a tendency towards higher LV dP/dt values than the placebo group. However, there were no differences between the groups in CO, HR, MAP; CVP or end diastolic pressure.

In the propranolol intoxication study, the levosimendan group had higher CO, LV dP/dt, SV, MAP and HR than the dobutamine and placebo groups ($P < 0.05$ for each time point) (Figure 9). There was no difference in SVR between the groups throughout the study.



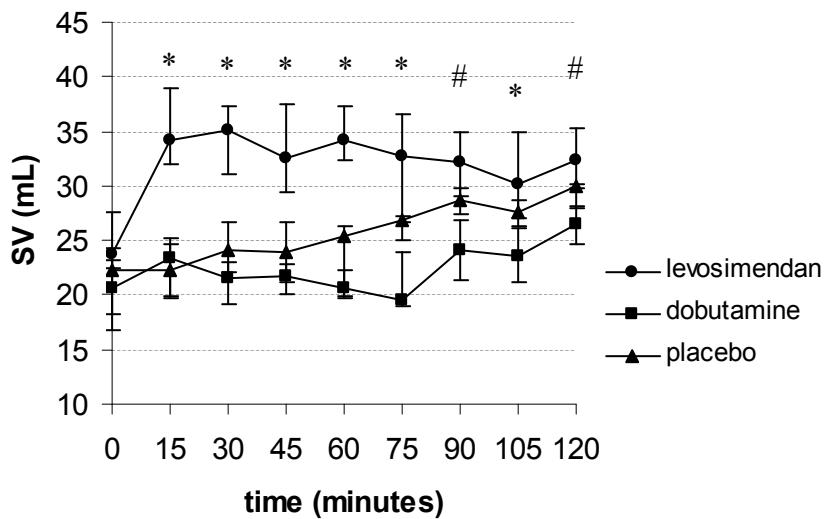
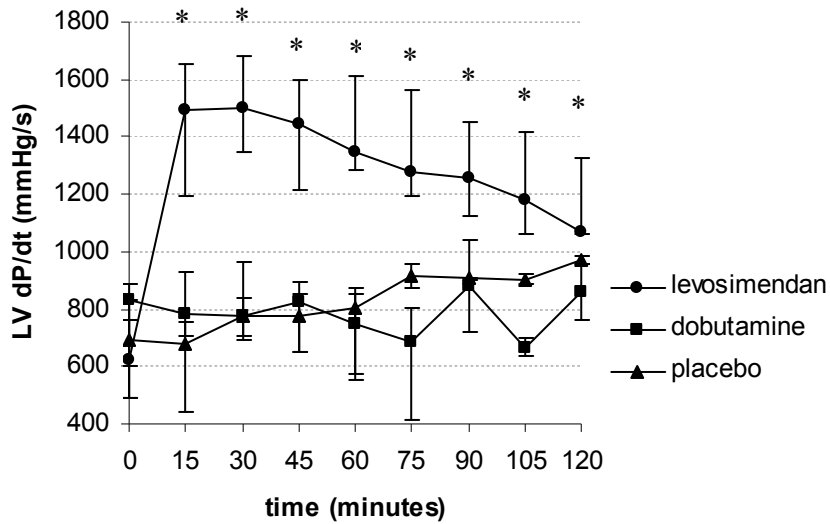


Figure 9. Haemodynamic changes in a porcine model of propranolol intoxication. Cardiac output (CO), maximum of the positive slope of the left ventricular pressure (LV dP/dt) and stroke volume (SV) between time 0 and time 120 in levosimendan, dobutamine and placebo groups (median and interquartile range; * $P < 0.05$ between levosimendan vs. placebo and dobutamine groups; # $P < 0.05$ between levosimendan and dobutamine groups).

5.3. Effect in splanchnic perfusion

Among the aortic surgery patients, the ΔPCO_2 gradient was lower in the levosimendan group after 60 minutes of aortic clamping [0.9 (0.6-1.2)kPa vs. 1.7 (1.1-2.1kPa)], ($P=0.002$) (Figure 10). Concomitantly, no statistical difference could be detected in ICG-PDR [29 (21-29)% vs. 20 (19-25)%], ($P= 0.055$) between the groups throughout the study period. There was no difference between the groups in gastric pH_i .

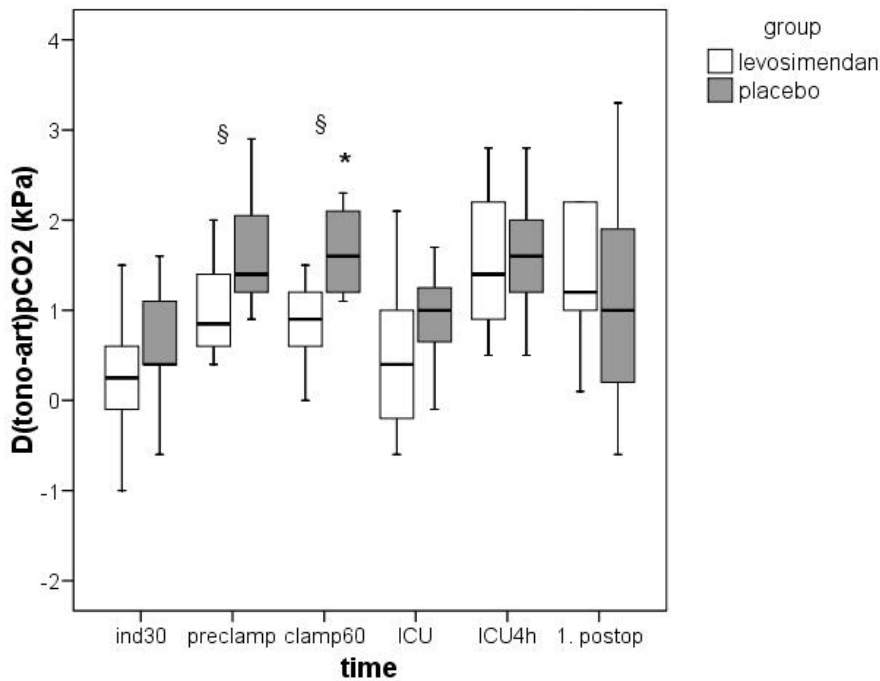


Figure 10. Gastric mucosal to arterial pCO_2 gradient in levosimendan (white columns) and placebo-treated (grey columns) patients at 30 min after induction of anaesthesia (ind30), before aortic clamping (pre-clamp), 60 min after aortic clamping (clamp60), on admission to intensive care unit (ICU), 4 h after admission (ICU4h) and on the first postoperative morning (1.postop). * $P<0.05$ by Wilcoxon's signed-rank test (against the baseline) and § $P<0.05$ by Mann-Whitney U-test (between the groups).

In the cardiac surgery patients, the hepatic vein base excess values were higher in the levosimendan group than in the control group right after surgery and 4 hours postoperatively (Figure 11). The hepatic vein saturation and lactate values were comparable between the groups throughout the study period. There was no difference in hepatic OER between the groups. The plasma bilirubin was within

normal range in both groups throughout the study although the bilirubin level was higher in the levosimendan group at baseline than in the control group 13 (SD=6) $\mu\text{mol/l}$ vs. 8 (SD=3) $\mu\text{mol/l}$ ($P=0.02$).

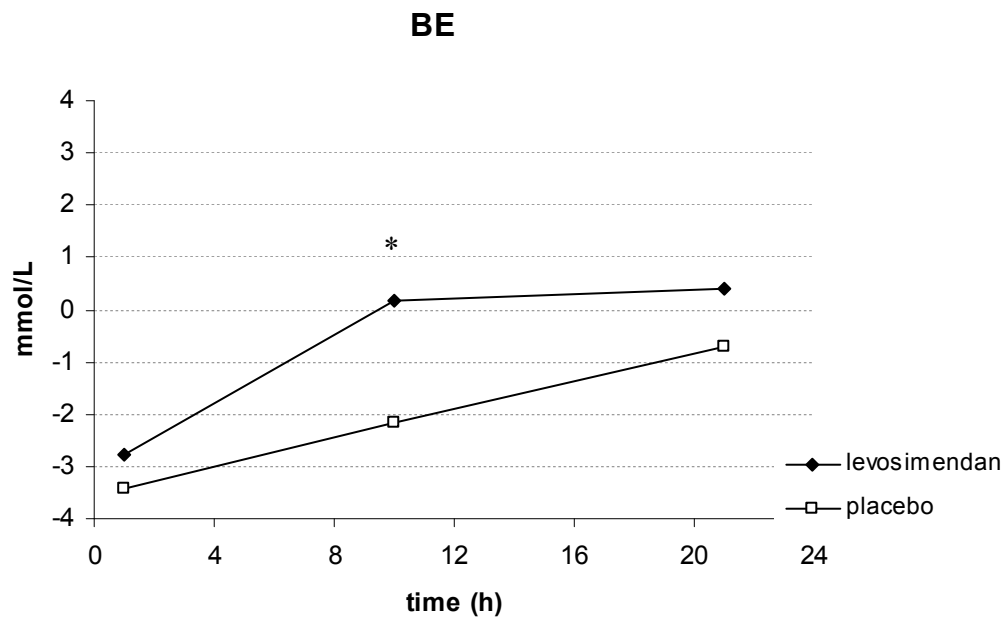


Figure 11. Mean hepatic vein BE immediately after induction of anaesthesia, four hours postoperatively and on the first postoperative morning. * $P<0.05$ between the groups.

5.4. Laboratory markers

In the aortic surgery patients there was no difference between the groups in the laboratory markers of haemoglobin, arterial lactate, SvO_2 , ScvO_2 , creatinine, P-CK-MBm or troponin T release.

In the cardiac surgery patients, levosimendan was rapidly absorbed and eliminated from plasma after the study drug infusion discontinued. Twenty-four hours after the start of the infusion the mean concentration was 30 (SD=20) ng/ml and this was below the lower limit of quantification (i.e. $< 0.200\text{ng/ml}$) at 48 hours. Both metabolites showed a rising trend until 96 hours after the start of the levosimendan infusion. The mean concentrations of OR-1855 and OR-1896 on day

4 were 8.4 (SD=3.3) ng/ml and 8.8 (SD=4.9) ng/ml respectively. At baseline, NT-proBNP was elevated in 10 patients in the levosimendan group and in 8 patients in the control group; 2088ng/l (SD=2541) vs. 1232ng/l (SD=1010), ($P=0.29$), respectively. Postoperatively, NT-proBNP increased in both groups on the fourth postoperative morning; 3329ng/l (SD=2662) in the levosimendan group vs. 3298ng/l (SD=1933) in the control group ($P=ns$). P-CK-MB, creatinine and systemic lactate values were similar between the groups throughout the study period. In two patients in both groups P-CK-MB exceeded 75U/l on the first postoperative morning indicating myocardial injury. By the second postoperative morning P-CK-MB was above 75U/l in one patient in the levosimendan group.

In the verapamil intoxication study there was no difference between the groups in SvO₂ or lactate values.

In the propranolol intoxication study, the final blood samples were taken immediately before the clinically estimated collapse of haemodynamics in each pig or at the end of the follow-up period of 120 minutes. In those blood samples there was a decrease in SvO₂ in both dobutamine [from 39 (38; 41) % to 22 (19; 40) %] and placebo [from 30 (26; 39) % to 22 (19; 34) %] groups compared with the levosimendan group, in which SvO₂ increased from 40% (32; 45) at drug 0 (when the intoxication was complete) to 56% (44; 63) at drug 120 (end of the follow-up period).

5.5. Mortality

In the clinical studies, the respective ICU, hospital and 6-month mortalities for all patients were 2.3% (1/44), 4.5% (2/44) and 9.1% (4/44). In the levosimendan treated patients one patient died in the ICU due to atherosclerotic heart disease, one patient in hospital due to unspecified chronic ischaemic heart disease and one patient during 6-month follow up due to subsequent myocardial infarction of unspecified site. In the placebo group one patient died due to pulmonary cancer during the 6-month follow up. All three patients who died from cardiac problems were female, the one patient who died for cancer was male. The 6-month mortality in the aortic surgery study was 10% (2/20) and in the cardiac surgery study 8.3% (2/24).

In the verapamil intoxication study, one pig died in the levosimendan group before completion of the experiment. Five out of six pigs died during the experiment in the control group. The animal survival is presented in Figure 12.

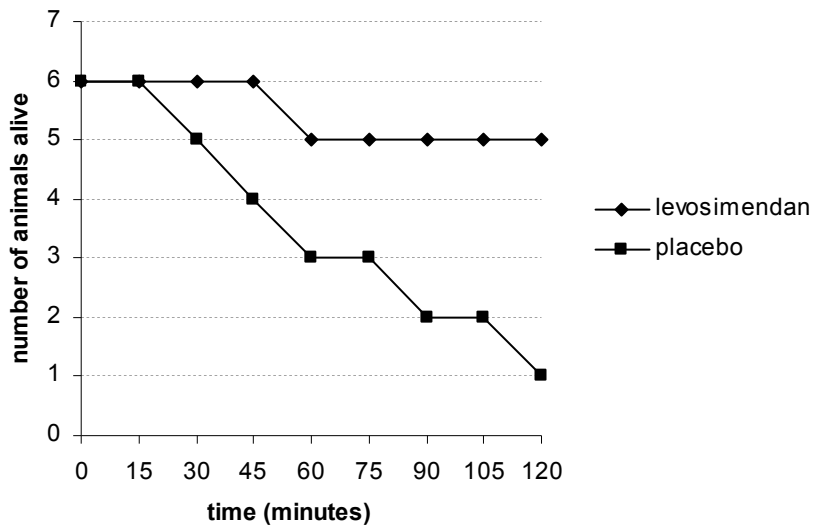


Figure 12. Pig survival after verapamil intoxication in levosimendan and placebo groups.

In the propranolol intoxication study, all pigs in the levosimendan group survived the experiment. By contrast, four out of six and seven out of nine pigs died during the experiment in the placebo and the dobutamine groups respectively (Figure 13).

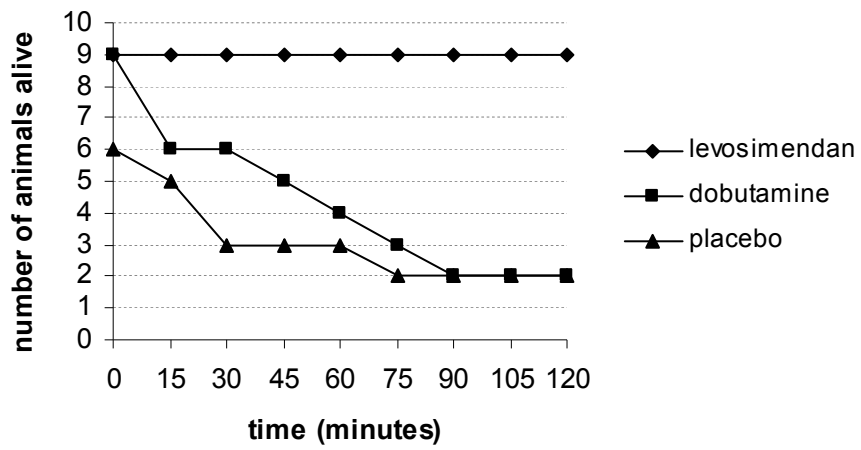


Figure 13. Pig survival between drug 0 (when the propranolol intoxication was complete and study drugs started) and drug 120 (end of follow-up time) in levosimendan, dobutamine and placebo groups.

6. Discussion

6.1. Effects on haemodynamic parameters

The haemodynamic effects of levosimendan on invasively measured variables were studied in all studies. Non-invasive methods (echocardiography and ICG_{WB}) were used only in cardiac surgery patients.

In the clinical studies (Studies I and II) CO increased in response to levosimendan even though HR remained constant. The trend towards better SV in the aortic surgery patients was supported by the significantly increased SI in cardiac surgery patients. Therefore the higher CO values in the levosimendan group were due to enhanced SI rather than an increase in HR.

The HR increased in the levosimendan group in both experimental studies (Studies III and IV). Some of the beneficial effect in CI may be due to an increase in HR, not only the improved SI in animals. The reason for the tachycardia in the animal studies could have been due to the study protocol, in which the levosimendan was administered to the animals in the presence of severe myocardial depression. The vasodilation was probable even though there were no significant differences in SVR between the study groups. It may have led to a decrease in preload causing reflectory tachycardia.

The duration of the haemodynamic effects after levosimendan infusion in the cardiac surgery patients has not been studied earlier. In the congestive heart failure patients the increase in CO was present for 12-13 days (Lilleberg et al., 2007). The results in cardiac surgery patients show that levosimendan, measured with ICG_{WB}, improved the haemodynamics for the four-day postoperative period when the study period was completed. The CI and SI remained significantly better in the levosimendan group than in the control group and there was no difference in HR between the groups. Levosimendan was able to support cardiac function, which deteriorated in the control group despite the normal systolic function at baseline.

Our finding concurs with those of the study on congestive heart failure patients by Lilleberg and co-workers, although we do not know how long the effect of levosimendan lasted in our study population. It is likely that the beneficial effects continued some additional days due to the finding that there was no fading in the difference in CI or SI between the groups on the fourth postoperative day. These observations suggest that levosimendan may prevent the myocardial dysfunction that often occurs after cardiac surgery (Kloner et al., 1994).

In the cardiac surgery study, diastolic dysfunction was evaluated by Doppler and tissue Doppler imaging. The diastolic function was assessed with E/E', which combines the influence of transmitral driving pressure and myocardial relaxation. De Luca et al. have shown a decrease in LV filling pressure assessed by E/E' after levosimendan treatment (De Luca et al., 2006). In our study the difference between the groups was not significant despite a decreasing trend in the levosimendan group and increasing trend in the control group. This may be due to the haemodynamic treatment protocol, which aimed at standardized filling pressures. The levosimendan group needed more fluids to achieve pre-set filling pressure. No statistically significant changes were seen in any other measurements concerning diastolic function. The left atrial volume index, peak Doppler velocities of early (E) and late diastolic (A) flow, deceleration time of E-wave, and E/A ratio did not differ significantly between treatment groups at any time point. Nevertheless, the SI increased, the LVEF remained constant, and diastolic function assessed with E/E' showed an increasing trend in the levosimendan group compared to the control group. This means that, in addition to improvement in systolic function, diastolic function also improves.

The results reported in this thesis suggest that the haemodynamic effects observed at the beginning of the levosimendan infusion derive from the parent drug itself. In the cardiac surgery study, in which the levosimendan infusion was initiated the day before surgery, the formation of the metabolites was measured and the concentrations showed an increasing trend until the fourth postoperative day. The concentrations on day 4 were higher than the peak concentrations in the study by Eriksson et al. where levosimendan was started immediately after induction of anaesthesia (Eriksson et al., 2009). This supports our hypothesis that the formation of the metabolites is not disturbed by possible hypoperfusion caused by CPB when levosimendan is started preoperatively. I hypothesize that the effects of

levosimendan are a sum of effects of the parent drug (short-term effects) and the metabolite OR-1896 (long-term effects).

6.2. Effects on splanchnic circulation

In our study, all patients had major vascular surgery or combined cardiac surgery. In all cardiac surgery patients the perfusion time was more than 80 minutes. Liver OER and ICG-PDR were used as indirect measures of the adequacy of the whole splanchnic perfusion. We detected no statistically significant difference between the levosimendan and placebo treated patients, which suggests that possibly the total splanchnic blood flow did not increase with levosimendan. In aortic surgery patients, a lower ΔPCO_2 gradient was observed in the levosimendan group. This may indicate that levosimendan limits gastric mucosal perfusion deficit during aortic cross-clamping. In cardiac surgery patients, the bilirubin had an increasing trend in the control group while it remained at baseline level in the levosimendan group. An indirect sign of better reserved hepatic blood flow during the cardiac surgery may be the higher hepatic vein BE level during the immediate postoperative period despite similar arterial BE levels. Hepatic venous congestion during weaning from CPB has been suggested to play a role in post-CPB related hepatic injury (Yamashita et al., 1998). If this is so, the right ventricular function is of great importance in preventing hepatic dysfunction. Levosimendan has also been shown to improve the haemodynamic parameters of right ventricular performance (Russ et al., 2009).

6.3. Laboratory markers

SvO₂ and ScvO₂ have been proposed as indicators of the oxygen supply / demand relationship. Moreover, low ScvO₂ values in the perioperative phase have been related to increased risk of postoperative complication in high-risk surgical patients

(Collaborative Study Group on Perioperative ScvO₂ Monitoring, 2006; Pearse et al., 2005). A mismatch between oxygen supply and demand has been associated with a prolonged stay in intensive care in cardiac surgery patients (Pölönen et al., 1997). There was no difference in either ScvO₂ (Study I) or SvO₂ values (Studies I and II) between the groups. This indicates that oxygen supply and demand in perioperative or early postoperative phase was similar in the levosimendan and placebo groups in aortic surgery and cardiac surgery patients.

In the experimental propranolol intoxication study the significant improvement in haemodynamics led to increased oxygen supply in the levosimendan group compared to the dobutamine or placebo groups. This was seen in the significant improvement of SvO₂ and it can be considered as a crude measure of the adequacy of perfusion at whole body level.

There was no significant difference in troponin T (Study I), P-CK-MBm (Study I), or P-CK-MB (Study II) between the groups. A recent meta-analysis suggests that levosimendan is associated with a reduction in cardiac troponin release in cardiac surgery patients (Zangrillo et al., 2009). The release of the injury markers we used did not confirm this finding.

The NT-proBNP concentration has been shown to be likely to predict death, hospitalizations and combined morbidity in HF patients (Latini et al., 2004). Levosimendan has been reported to produce a rapid decrease in natriuretic peptides in several studies on HF patients (Lilleberg et al., 2007; Mebazaa et al., 2009). In our Study II, the NT-proBNP was similar between the groups. The same trend in NT-proBNP was seen by Eriksson et al. in their study on cardiac surgery patients (Eriksson et al., 2009) indicating that there was similar myocardial stretch and diastolic pressure load in the levosimendan treated patients to that in the control group. This was probably because, in the treatment protocol of both studies, fluids were infused to meet the PCWP goal with similar filling pressures.

6.4. Mortality

Patient mortality in Studies I and II was similar to that reported in earlier studies (Lepántalo et al., 2008; Kramer and Zimmerman, 2008; Biancari et al., 2010). The small sample size does not permit comparison of the differences in mortality between the levosimendan and placebo groups. It would take approximately 2,500 CABG + AVR patients with per group to detect a 25% relative risk reduction in mortality rates between the treatment groups, assuming that the 6- month mortality is 8% with 80% power ($\alpha = 0.05$) (Dupont and Plummer, 1990).

However, levosimendan improved survival in both verapamil and propranolol-induced experimental myocardial depression.

In the propranolol intoxication study (Study IV) the survival benefit in the levosimendan treated animals was associated with better preserved myocardial function measured in CO, SV, MAP and LV dP/dt.

In the verapamil intoxication (Study III), however, the survival benefit in the levosimendan group was seen against placebo but only a tendency to improved cardiac function could be detected. However, earlier experimental studies have reported no effect on or decrease in survival and haemodynamics when levosimendan is compared to placebo or active treatment (Graudins et al., 2008; Abraham et al., 2009; Graudins and Wong, 2010). There are two previous case reports which also recommend the use of levosimendan in calcium channel blocker poisoning (Varpula et al., 2009; Osthoff et al., 2010).

6.5. Strengths and weaknesses of the study

The reasons for this study were the problems clinicians face in their everyday practice. The studies reported in this thesis were intended to investigate four of these problems: splanchnic hypoperfusion, insufficient cardiac function in cardiac surgery patients, calcium channel blocker poisoning, and β - blocker intoxication. We found evidence that levosimendan may be useful for clinicians in three of these situations.

First, major surgery has been reported to cause impaired splanchnic perfusion leading to high mortality and increased cost of care. Earlier studies have not found an ideal inodilator with splanchnic vasodilation. Studies I and II tried to find a new

treatment to prevent gastrointestinal hypoperfusion and partly succeeded in demonstrating the positive effect of levosimendan.

Second, preoperative infusion of levosimendan provides sustained improved haemodynamics in cardiac surgery patients and the formation of active metabolite was more efficient than that reported in earlier studies (Study II). This may influence the starting time of levosimendan in clinical practice.

Third, levosimendan may be a new promising alternative in the treatment of severe β - blocker poisoning (Study IV).

The main limitations of the studies are as follows:

Study I: This study illustrates the difficulties associated with clinical studies on splanchnic circulation. This vascular bed is difficult to investigate, with no simple obvious method.

Gas tonometry has been shown to have methodological problems, such as variation in data quality (Creteur et al., 1999). To minimize this problem, the authors tried not to compare absolute values but trends between the groups.

Concerning the ICG-PDR method, there are no references comparing ICG-PDR to directly measured splanchnic blood flow. There could be an increase in splanchnic perfusion with levosimendan, but the method might not be sensitive enough to measure changes in splanchnic perfusion, especially given the small number of patients in our study.

Study II: CPB may impair intestinal function. The production of the intermediate metabolites of levosimendan, OR-1855 and OR-1896, may decrease if levosimendan is given perioperatively as opposed to an infusion on the day before surgery as in our study. The questions on the levels of the metabolites and possible changes in the haemodynamic variables as a result of pre- and perioperative administration of levosimendan remain unanswered. To investigate this hypothesis, levosimendan 24 hours before cardiac surgery should be directly compared with the administration of levosimendan during the operation. Thus, our Study II would have needed a group treated with intraoperative levosimendan infusion.

Study III: Levosimendan was compared with no treatment under conditions which resulted in more than 80% mortality if left untreated. Under such circumstances, any cardiovascular support might have improved survival. This study does not answer the question whether levosimendan is beneficial in calcium channel intoxication when compared to standard treatment. Moreover, twelve animals is a

small number in an experiment trying to ascertain the mortality rates of two different treatments.

Study IV: The effect of levosimendan was compared to placebo and dobutamine treatments. However, this study does not establish levosimendan as superior or equivalent to standard therapy, since dobutamine is not the recommended as the treatment of choice for severe β -blocker intoxication (Newton et al., 2002). Therapies such as calcium or glucagon would have been good choices as alternatives to dobutamine, especially regarding the mortality. In this study, the focus was mostly on myocardial contractility.

6.6. Clinical aspects and future perspectives

Levosimendan was designed as an inotrope for the treatment of cardiac failure. Relatively soon it was realized that the inotropic property could not explain all the haemodynamic effects the drug has. It also causes vasodilation. This reduces preload and afterload and has been demonstrated to occur in both arterial and venous vascular beds. Vasodilation can be both beneficial and deleterious causing hypotension but also enhancing blood flow and oxygen supply in different vascular beds. This feature was studied in our Studies I and II and also by others but it is still not fully understood and needs more research. New patient groups, for example peripheral vascular surgery patients with critical ischaemia, might benefit from the vasodilating effect of levosimendan. However, there are no studies on this issue in the literature.

Overactivation of inflammatory cascades is involved in the pathogenesis of sepsis. The potential anti-inflammatory effects and the beneficial effects on the mitochondrial function of levosimendan rise an interesting alternative, at least in the treatment of septic cardiomyopathy. The research finding the optimal trigger for initiating inotropes (levosimendan among others) for the treatment of septic myocardial dysfunction has been intensive but is not yet conclusive (Varpula et al., 2005).

In Finland, the cost per patient for a 24-h infusion of levosimendan is approximately 720€. In ADHF patients, the overall costs of using dobutamine were similar to those of levosimendan due to a shorter length of stay in the ICU even though the former drug costs were less than a half of the cost of levosimendan (Oliveira et al., 2005). Similar results were achieved when the economic analysis compared levosimendan to local standard of care (de Lissovoy et al., 2010). However, in our study, there were no differences in length of either ICU or hospital stay.

Due to the high cost of levosimendan and pressure from hospital administrators to save money, levosimendan is often reserved as a last resort therapy. This strategy may be late to gain the optimal effect in reversing the vicious circle of a critically ill patient. Studies investigating the optimal timing of administering levosimendan including economic analysis are warranted.

The current recommendation for the duration of levosimendan infusion is 24 hours. However, there is some clinical experience with cardiac surgery patients that there is a short decrease in the cardiac function immediately after the end of infusion in some patients. The cardiac function improves in a few hours probably due to the increasing amount of metabolite OR-1896 (clinical experience from Study II). There are two ways to avoid cardiac deterioration. In our Study II, levosimendan was given preoperatively so there was time for OR-1896 to form. Another alternative could be a longer than 24-hour infusion of the parent drug levosimendan.

The only current indication for the use of levosimendan is the treatment of severe acutely decompensated heart failure. However, levosimendan could be more widely applicable in perioperative and critical care settings. Large randomized trials are warranted with the use of levosimendan in these situations. The encouraging results in sepsis, cardiogenic shock and cardiac surgery need to be confirmed before any evidence-based recommendations can be made. Future studies on non-cardiac surgery patients with heart and renal insufficiency, pulmonary hypertension, viral myocarditis, and renal and hepatic blood flow are warranted. A controlled, randomized study investigating beta blocker intoxication treated with levosimendan is not feasible but case reports would be most welcome. The inotropic support in patients treated with therapeutic hypothermia after CPR would need a clinical, randomized study.

7. Conclusions

Based on these studies the following conclusion can be drawn:

1. Levosimendan may have positive effects on gastric wall and hepatic blood flow. However, levosimendan seems not to favour total splanchnic perfusion. During surgery until 60 min after aortic clamping levosimendan increased cardiac output while no statistical differences in stroke volume could be detected.

2. Preoperatively infused levosimendan improves cardiac function for four postoperative days in cardiac surgery patients. The production of the intermediate metabolites of levosimendan, OR-1855 and OR-1896, may increase if levosimendan is given preoperatively as opposed to a perioperative infusion.

3. Levosimendan may improve survival in the experimental model of calcium-channel poisoning.

4. Levosimendan offers a promising new approach for the treatment of severe β -blocker poisoning.

Acknowledgements

I would like to express my gratitude to my two supervisors, Professor Leena Lindgren and Professor Esko Ruokonen. Leena, a scientist and teacher full of ideas and energy, patiently guided me to the world of science. Your door was always open to give advice on problems big and small. Esko, a visionary of intensive care, introduced me to the Finnish intensivist community and gave me an unforgettable glimpse of how an experienced and skilled critical care doctor works.

I thank the reviewers, Docent Kai Kiviluoma and Docent Mika Valtonen, for their excellent comments and criticism regarding this thesis.

I particularly acknowledge all the co-authors of the original publications. Especially, I want to thank Kati Järvelä and Jouni Kurola. Kati, this work would have been much harder without your advice, example and friendship. Giving up does not belong to your vocabulary. Jouni - tekemisen meininki. I think that is the best way to describe our collaboration.

I thank Sari Karlsson for sharing not only her office and vast clinical experience but also my anxieties and joys as a novice in scientific work. Aira Etelämäki and Michael Rorarius: you made it possible for me to complete this work. Your ability to organize the work in the operating rooms gave me an opportunity to do scientific work even during working hours.

I wish to thank study nurses Elina Halonen, Heikki Ahonen, Minna-Liisa Peltola and The Boys, and Pirjo Järventausta for their technical help in the clinical phases of the studies. Working with you was extremely uncomplicated and efficient.

Finally, I dedicate this work to my family: to my husband Poika Isokoski, not just for his patience during the time I spent with this work, you were the dictionary and the first reviewer of the drafts of the groping manuscripts. Most importantly, you let me make my own mistakes, which were the most important part in this learning process. To my son Valo, for being the light and enjoyment of my life. Your presence reminds me every day what is really important in life. You are lucky to

have grandparents that came to help every time when needed during the writing of this painful but exhilarating dissertation.

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Effects of levosimendan on indocyanine green plasma disappearance rate and the gastric mucosal–arterial pCO₂ gradient in abdominal aortic aneurysm surgery

H. LEPPIKANGAS¹, J. J. TENHUNEN¹, L. LINDGREN¹, J.-P. SALENIUS¹ and E. RUOKONEN²

¹Critical Care Medicine Research Group, Departments of Critical Care Medicine, Anesthesiology and Vascular Surgery, Tampere University Hospital, Tampere, Finland and ²Department of Intensive Care, Kuopio University Hospital, Finland

Background: Levosimendan has a dual mechanism of action: it improves myocardial contractility and causes vasodilatation without increasing myocardial oxygen demand. In a laboratory setting, it selectively increases gastric mucosal oxygenation in particular and splanchnic perfusion in general. The aim of our study was to describe the effects of levosimendan on systemic and splanchnic circulation during and after abdominal aortic surgery.

Methods: Twenty abdominal aortic aneurysm surgery patients were randomized to receive either levosimendan ($n = 10$) or placebo ($n = 10$) in a double-blinded manner. Both the mode of anaesthesia and the surgical procedures were performed according to the local guidelines. Automatic gas tonometry was used to measure the gastric mucosal partial pressure of carbon dioxide. Systemic indocyanine green clearance plasma disappearance rate (ICG-PDR) was used to estimate the total splanchnic blood flow.

Results: The immediate post-operative recovery was uneventful in the two groups with a comparable, overnight length of stay in the intensive care unit. Cumulative doses of additional vasoactive drugs were comparable between

the groups, with a tendency towards a higher cumulative dose of noradrenaline in the levosimendan group. After aortic clamping, the cardiac index was higher [4(3.8–4.7) l/min/m² vs. 2.6(2.3–3.6) l/min/m²; $P < 0.05$] and the gastric mucosal–arterial pCO₂ gradient was lower in levosimendan-treated patients [0.9(0.6–1.2) kPa vs. 1.7(1.2–2.1) kPa; ($P < 0.05$)]. However, the total splanchnic blood flow, estimated by ICG-PDR, was comparable [29(21–29)% vs. 20(19–25)%; NS]. Organ dysfunction scores (sequential organ dysfunction assessment) were similar between the groups on the fifth post-operative day.

Conclusion: Levosimendan favours gastric perfusion but appears not to have a major effect on total splanchnic perfusion in patients undergoing an elective aortic aneurysm operation.

Accepted for publication 31 January 2008

Key words: inotropy; calcium sensitizer; levosimendan; splanchnic perfusion; gastrointestinal tract; blood flow.

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GASTROINTESTINAL hypoperfusion may play an important role in the pathophysiology of overall tissue hypoperfusion abnormalities in major surgery, causing high mortality and increased cost of care (1). Impaired splanchnic perfusion may induce systemic inflammation and multiple-organ dysfunction (MOD). Perioperative bowel ischaemia and a significant increase in colonic epithelial apoptosis have been shown to occur as a response to open abdominal aortic surgery (2, 3). During abdominal aortic surgery, gut mucosal perfusion and colonic tissue flow decrease as measured by gastric tonometry and laser Doppler flowmetry (4). Previous attempts to find an ideal inodilator with preferential splanchnic vasodilatation have failed,

and the effects of various vasoactive agents on total splanchnic, or selectively, on gastric perfusion are inconsistent (5, 6).

Levosimendan is a rather new calcium-sensitizing inodilator that enhances myocardial contractility (7) and produces both coronary and peripheral vasodilatation (8) without increasing the myocardial oxygen demand (9). Previously, levosimendan has been used in patients suffering from congestive heart failure (10), post-CABG and in septic shock (11). In an experimental setting in anaesthetized intact dogs, levosimendan appears to be superior to milrinone and dobutamine in increasing gastric mucosal oxygenation selectively without requiring major increases in systemic oxygen demand or

delivery (12). It favours splanchnic (total splanchnic, liver and renal) circulation under normal physiological conditions (13) and in experimental endotoxin shock (14–16). To date, the only limited clinical human data are available on the effects of levosimendan during major surgery, post-CABG surgery (17) or in septic shock in general or on regional perfusion (18). In post-CABG patients, levosimendan increased the cardiac output and stroke volume and decreased vascular resistance without increasing oxygen consumption (17). The clinical efficacy of levosimendan as an alternative to increasing the dose of dobutamine in septic shock has been shown by Morelli et al. (18).

We hypothesized that levosimendan could improve splanchnic blood flow and tissue perfusion perioperatively in major vascular surgery. To test our hypothesis, we measured the effect of levosimendan on splanchnic blood flow and tissue perfusion in patients undergoing elective aortic aneurysm surgery.

Methods

Patients

The Local Ethics Committee approved the study protocol. Patients with an infrarenal abdominal aortic aneurysm undergoing elective surgery were enrolled after written, informed consent. We excluded patients with aortic valvular and occlusive disease or known sensitivity for an adverse reaction to levosimendan. We randomized the patients into a placebo ($n = 10$) or a levosimendan ($n = 10$) group. Randomization (sealed opaque envelopes) was performed by the pharmacist of the satellite pharmacy at the department of intensive care unit (ICU). The pharmacist also prepared and diluted the study drugs thereby ensuring that the investigators, surgeons, anaesthesiologists, research assistants and medical and nursing staff in the ICU and on the ward were blinded to the group assignment.

Mode of anaesthesia, fluid therapy and use of vasoactive drugs

A radial arterial cannula and a pulmonary artery catheter (Arrow[®], Reading, PA, USA) were inserted under local anaesthesia before the operation to enable continuous haemodynamic monitoring. An epidural catheter (Portex[®], Watford, UK) was inserted at the thoracolumbal T12–L1 intervertebral space using a standard loss of resistance technique under local anaesthesia for post-operative pain relief,

after which the baseline measurements were made. The patients in the treatment group received a levosimendan (Simdax[®], Orion Pharma, Finland) 24 µg/kg i.v. bolus over 30 min before induction of anaesthesia. The control patients received a placebo (thiamin-coloured 5% glucose) infusion of equivalent volume over the same time interval. The levosimendan infusion 0.2 µg/kg min or placebo infusion was continued for 24 h.

The anaesthesia was induced with propofol (1.5–2.5 mg/kg according to clinical needs), fentanyl (2–3 µg/kg) and cisatracurium (0.15 mg/kg). After tracheal intubation, the lungs were mechanically ventilated with an oxygen–air mixture (FiO₂ 0.40–0.60 to maintain peripheral SpO₂ over 92%). Anaesthesia was maintained with an infusion of propofol adjusted to maintain state entropy (SE) levels between 40 and 60, the target being 50 (Entropy Sensor[®], GE Healthcare, Milwaukee, WI, USA) and fentanyl (1–2 µg/kg bolus) combined. Neuromuscular blockade was achieved with bolus injections of cisatracurium according to clinical needs. It was monitored with ulnar nerve train of four (TOF) stimulation and neuromuscular transmission monitoring (TOF-Watch[®], Organon, the Netherlands). The tidal volumes were adjusted in the operating theatre and post-operative unit according to the ARDS network guidelines of 6–8 ml/kg of ideal body weight (19). The use of dobutamine was aimed to maintain the cardiac index >2.0 l/min/m². Mean arterial pressure (MAP) was maintained >65 mmHg using norepinephrine. Before any use of inotropes or vasoconstrictors, the intravascular volume was ensured by adjusting pulmonary artery occlusion pressure (PAOP) to 8–12 mmHg. Mixed venous oxygen saturation was maintained >70% by infusing crystalloids, colloids and packed red blood cells to maintain the haemoglobin concentration over 80 g/dl.

Surgical procedure

The surgical procedure was standardized. The same individual senior surgeons were chosen to minimize the confounding effect of differences in the surgical technique and skills. The abdominal aorta was exposed through a full midline laparotomy. An infrarenal clamp was accomplished under systemic heparinization (dose 5000 IU). The aortic occlusion time was recorded. A dacron Y-prosthesis was used to reconstruct the aortic and iliac arteries. After the anastomoses were prepared, the lower trunk blood circulation was

returned, careful haemostasis was ensured and the laparotomy was closed in layers.

Haemodynamic monitoring

The heart rate, MAP, central venous pressure (CVP), PAOP and cardiac output were recorded. (HP, Palo Alto, CA, USA until 9/2005 then Philips, Amsterdam, the Netherlands). The derived cardiovascular variables (cardiac index and systemic vascular resistance) were calculated using standard formulae. Cardiac output was measured by bolus injectates in triplicate using 10 ml of room-temperature 0.9% sodium chloride. Haemodynamic data were collected at the following time points: at baseline, 30 min after induction of anaesthesia, before aortic clamping, 60 min after clamp, on admission to the ICU, 4 h post-ICU admission and 24 h after levosimendan/placebo bolus. Standard 12-lead ECG recordings were obtained perioperatively before surgery, immediately post-operatively and after 20–24 h on the first post-operative day.

Post-operative pain treatment

The epidural infusion (bupivacain 0.25%+NaCl 0.9% 26 ml+fentanyl 200 µg 2–7 ml/h) with per oral paracetamol was started at arrival on ICU.

Tonometry and indocyanine green plasma disappearance rate

To assess a surrogate for gastric mucosal perfusion, mucosal pCO₂ was measured and gastric mucosal to arterial pCO₂ gradient was calculated during the perioperative period by a gastric tonometer (Tonocap[®], Datex, Ohmeda, Finland). The tonometer catheter was inserted after the induction of anaesthesia, and the correct placement in the stomach was confirmed manually in the beginning of surgery and by chest X-ray obtained in the ICU. Total splanchnic blood flow was estimated by measuring the indocyanine green plasma disappearance rate (ICG-PDR) transcutaneously (20, 21). Briefly, each patient was connected to an ICG finger clip, which was connected to a liver function monitor (LiMon[®], Pulsion Medical Systems, Germany). A Dose of ICG 0.25 mg/kg (22) was injected through a central venous line of the pulmonary artery catheter at baseline, before and during aortic clamping and post-operatively. Arterial, central venous and mixed venous blood-gas tensions and oxygen saturations, haemoglobin and lactate concentrations were measured before induction of

anaesthesia, before and during aortic occlusion and at the end of surgery. Troponine T concentrations were measured at admission to the ICU and at the first post-operative morning.

Primary end point parameters

The primary endpoint parameters were cardiac index and stroke volume index. Surrogates for total splanchnic perfusion and gastric wall perfusion were ICG-PDR and gastric mucosal-arterial pCO₂ gradient. Mortality, length of ICU stay and morbidity were considered to be secondary endpoints.

Data analysis and statistics

ICG-PDR was a rather new method and this is why we did not have enough data for sample size calculations. Instead, we used splanchnic blood flow and mucosal pH as surrogates. We assumed an SD of 0.21/min/m² for splanchnic blood flow and 0.068 for mucosal pH and a within-subject coefficient of variation of 4% for splanchnic blood flow measurements (23, 24). Using a two-sided α of 0.05, this would allow us to detect a change of 0.18 litre/min/m² in splanchnic blood flow and 0.06 pH units in mucosal pH with 80% power with 10 evaluable patients per group. One patient was excluded from post-operative analyses but was included for perioperative data. The group allocation was unblinded for this patient (placebo) because of profound post-operative oozing. Another patient was randomized to supplement the sample size.

The majority of the data was normally distributed as tested by the Kolmogorov–Smirnov test. Based on the kurtosis and skewness of the data, non-parametric statistical tests were used. Data are therefore presented as median (25–75th percentiles). We used Friedman two-way analysis of variance. When the Friedman test was found to be significant, the Wilcoxon signed-rank test was used to compare the values at baseline vs. each time point. Two-tailed tests were used and the Bonferroni correction was not applied. Comparably, when significant, post-Friedman analysis between the groups was performed by the Mann–Whitney *U*-test. The χ^2 -test was used to compare the need for additional vasoactive drugs in the two groups. SPSS software (SPSS[™] 15.0 for Windows, SPSS Inc., Chicago, IL, USA) was used for all statistical analyses. A *P*-value of <0.05 was considered to be significant.

Results

The patient characteristics were comparable in the two groups (Table 1). Six patients in the placebo

Table 1

Patient characteristics in levosimendan ($n = 10$) and placebo-treated groups ($n = 11$ at the baseline).

	Placebo	Levosimendan
Age (years)	72 (61; 74)	64 (60; 72)
Sex (M/F)	10/1	6/4
Weight (kg)	75 (65; 78)	80 (69; 94)
DM	0	2
HTA	7	7
COPD	3	1
CAD	2	1
β -blockers	6	4
SOFA post-operative day 1	3 (2.25; 4)	3.5 (3; 4.75)
SOFA post-operative day 5	1 (0.5; 1)	0 (0; 0)
Duration of surgery (min)	213 (191; 254)	223 (183; 235)
Blood loss (ml)	2175 (1200; 3075)	1300 (1050; 2000)

DM, diabetes mellitus; HTA, hypertonia; COPD, chronic obstructive pulmonary disease; CAD, coronary artery disease; SOFA, sequential organ dysfunction assessment.

group and four patients in the levosimendan group used β blockers before surgery. One patient in the levosimendan group required intensive care for 6 days because of post-operative bleeding, two reoperations for haemostasis and development of non-dialysis-dependent acute renal failure. One patient in the placebo group and one in the levosimendan group required intensive care for 2 days because of oliguria and respiratory failure, respectively. Otherwise, the post-operative recovery was uneventful in both groups with an overnight post-operative period in ICU. One patient was excluded from further statistical analysis after the code was opened because of clinical judgement necessary for profound post-operative bleeding.

The peri- and post-operative haemodynamic characteristics are shown in Table 2. At baseline, before surgery, there were no differences between the levosimendan and the control groups in haemodynamic variables or splanchnic blood flow. During surgery until 60 min after aortic clamping, the cardiac index was higher in levosimendan-treated patients (Fig. 1a). Levosimendan was associated with a stable heart rate while in the placebo group the heart rate decreased from baseline to 60 min after aortic clamping. Stroke volume remained comparable in the two groups (Table 2, Fig. 1b). The gastric mucosal to arterial $p\text{CO}_2$ gradient was lower in the levosimendan group after 60 min of aortic clamping [0.9 (0.6–1.2) kPa vs. 1.7 (1.1–2.1) kPa; $P = 0.003$] (Fig. 2a). Concomitantly, ICG-PDR [29 (21–29)% vs. 20 (19–25)%; $P = 0.055$] was comparable between the groups

throughout the study period (Fig. 2b). There was no difference in gastric $p\text{H}_i$ between groups.

Six patients in the levosimendan group and three patients in the placebo group received norepinephrine during the operation ($P < 0.05$). Post-operatively, four patients in the levosimendan group, but none in the placebo group received norepinephrine ($P < 0.05$). Cumulative doses of norepinephrine were 1.5 $\mu\text{g}/\text{kg}$ (0; 6.3) and 0 $\mu\text{g}/\text{kg}$ (0; 0.9) in the levosimendan and the placebo groups, respectively ($P = 0.12$). Dobutamine was needed for two patients in the placebo group perioperatively and for one patient in both groups post-operatively to maintain sufficient cardiac performance. Two patients in the levosimendan group and four patients in the control group received sodium nitroprusside to control systemic blood pressure in the operating theatre, and one patient in both groups post-operatively. Neither ST-segment changes nor troponin T release were detected in either group post-operatively. One patient receiving levosimendan died during hospitalization because of acute exacerbation of severe COPD.

Discussion

The main findings of our study were: firstly, levosimendan increased cardiac output while stroke volume remained unchanged. Secondly, the patients receiving levosimendan needed norepinephrine more often for systemic hypotension. Thirdly, contrary to our hypothesis, levosimendan increased systemic blood flow but the total splanchnic blood flow did not increase as estimated by the PDR of indocyanine green dye (PDR %/min). Fourthly, even though the total splanchnic blood flow did not increase, the gastric mucosal to arterial $p\text{CO}_2$ gradient remained lower in the levosimendan group, suggesting higher gastric mucosal perfusion.

A previous report by Morelli et al. (18) shows that levosimendan increased gastric mucosal perfusion in patients with septic myocardial depression, decreased the $p\text{CO}_2$ gradient and increased capillary blood flow measured by laser Doppler flow. A decreased $p\text{CO}_2$ gradient was similarly seen in our study and by Morelli et al., who also demonstrated increased gastric capillary blood flow. This may show that also in our study gastric mucosal perfusion increased rather than e.g., the Haldane effect (25). Regional perfusion heterogeneity has been described previously by several

Levosimendan favors gastric but not total splanchnic perfusion in major vascular surgery

Table 2

Haemodynamic characteristics in levosimendan ($n = 10$) and placebo-treated groups ($n = 11$ from the baseline until clamp60, $n = 10$ thereafter).

	Baseline	Pre-clamp	Clamp 60	ICU 4 h	Pop 1	P-value, Friedman
HR (beats/min)						
Levosimendan	70 (64, 74)	74 (65, 81)†	73 (66, 77)†	83 (69, 95)	90 (86, 102)*, †	0.003
Placebo	60 (59, 62)	53 (51, 59)*	53 (47, 59)*	65 (55, 76)	78 (72, 82)*	0.000001
MAP (mmHg)						
Levosimendan	95 (83, 107)	82 (70, 84)*, †	74 (68, 78)*	70 (63, 76)*	70 (66, 78)*	0.00002
Placebo	99 (88, 105)	91 (87, 98)	78 (71, 85)*	82 (66, 88)*	79 (69, 93)*	0.000001
CVP (mmHg)						
Levosimendan	7 (5, 8)	9 (7, 10)	9 (8, 10)	7 (4, 8)	4 (1, 8)	0.00007
Placebo	6 (3, 8)	9 (6, 13)*	9 (7, 14)*	5 (4, 6)	3 (1, 4)	0.000003
PAOP (mmHg)						
Levosimendan	10 (7, 11)	12 (10, 14)	12 (11, 13)	9 (7, 13)	7 (6, 11)	0.001
Placebo	11 (6, 12)	13 (10, 17)	11 (9, 16)	9 (5, 11)	9 (5, 10)	0.053
SVRI (dyne/s cm ⁵ m ²)						
levosimendan	2160 (1960, 2502)	1252 (1052, 1772)*, †	1284 (1052, 1313)*, †	1398 (1137, 2005)*	1356 (1137, 1562)*, †	0.009
Placebo	2153 (1788, 2364)	2302 (2125, 2529)	2231 (1444, 2744)	2049 (1688, 2213)	1730 (1650, 1956)*	0.027
SvO ₂ (%)						
Levosimendan	73 (71, 76)	77 (73, 80)	71 (68, 76)	67 (64, 71)*	65 (57, 71)*	0.0003
Placebo	75 (71, 80)	76 (71, 80)	72 (68, 76)	65 (59, 72)*	70 (66, 75)	0.003
Hb (g/l)						
Levosimendan	121 (118, 129)	101 (89, 104)*	76 (67, 80)*	87 (80, 98)*	95 (88, 99)*	<10 ⁻⁶
Placebo	121 (118, 124)	102 (92, 102)*	62 (59, 71)*	84 (73, 96)*	95 (86, 96)*	<10 ⁻⁶
Arterial lactate (mmol/l)						
Levosimendan	0.6 (0.5; 1.1)	0.8 (0.5; 1.3)	1.0 (0.6; 1.9)*	1.3 (1.0; 1.9)*	0.7 (0.5; 1.0)	0.001
Placebo	0.7 (0.5; 0.8)	0.6 (0.6; 0.7)	0.6 (0.6; 0.8)	1.2 (1.0; 1.8)*	0.6 (0.5; 1.0)	<10 ⁻⁶
PVRI (dyne/s cm ⁵ m ²)						
Levosimendan	213 (129, 230)	152 (114, 218)	228 (155, 303)	203 (194, 260)	199 (141, 302)	0.48
Placebo	193 (114, 251)	174 (101, 229)	131 (66, 198)	205 (142, 236)	210 (91, 293)	0.86
Ph _i						
Levosimendan	7.38 (7.34; 7.44)‡	7.30 (7.27; 7.34)	7.28 (7.27; 7.33)	7.27 (7.23; 7.28)	7.31 (7.26; 7.35)	0.002
Placebo	7.40 (7.34; 7.40)‡	7.27 (7.24; 7.32)	7.28 (7.21; 7.32)	7.26 (7.23; 7.28)	7.33 (7.30; 7.36)	0.001
Temperature (°C)						
Levosimendan	36.4 (36.1; 36.5)	35.6 (35.1; 35.8)*	35.8 (35.2; 35.9)*	36.9 (36.1; 37.1)	37.5 (36.7; 37.7)*	<10 ⁻⁶
Placebo	36.2 (36.1; 36.2)	35.3 (35.2; 35.8)*	35.2 (35.1; 35.8)*	36.9 (36.5; 37.2)	37.8 (37.5; 37.9)*	<10 ⁻⁶

HR, heart rate; MAP, mean arterial pressure; CVP, central venous pressure; PAOP, pulmonary artery occlusion pressure; SVRI, systemic vascular resistance index; SvO₂, mixed venous oxygen saturation; Hb, hemoglobin; PVRI, pulmonary vascular resistance index.

* $P < 0.05$ by Wilcoxon's signed-rank test (against the baseline) and

† $P < 0.05$ by Mann-Whitney U -test (between the groups).

‡ 30 min after induction of anaesthesia.

authors in an experimental (26, 27) or a clinical setting (5). Gastric mucosal perfusion changes do not reflect changes in other parts of the GI tract (27) or in the splanchnic region overall. In our study, ICG-PDR did not increase even with an increased cardiac index. This could imply that splanchnic blood flow did not increase in parallel with increasing cardiac index. However, the literature supporting the idea that ICG-PDR is a reliable surrogate for total splanchnic blood flow is limited. A fair conclusion might be that levosimendan was not associated with an enhanced hepatic blood flow/metabolism under this clinical condition. Concomitantly, with increasing cardiac index, we

observed a lower gastric mucosal to arterial pCO₂ gradient in the levosimendan-treated patients. This may imply that indeed, levosimendan attenuates or limits gastric mucosal perfusion deficit during aortic cross-clamping. Morelli et al. (18) found that levosimendan improved systemic and regional perfusion in patients with a septic cardiac dysfunction under conditions where 5 µg/kg/min of dobutamine was no longer efficacious. Levosimendan increased gastric mucosal flow (reduction in P_{g-a}CO₂), creatinine clearance and urinary output while it decreased lactate concentrations. They did not measure or estimate the total splanchnic blood flow.

Cardiac output was increased in response to levosimendan even though the heart rate remained unchanged. Therefore, there could be an increase in stroke volume, even though a statistical difference

was not detected probably due to the small number of patients. The stability in heart rate in the levosimendan group is also noteworthy compared with the trend towards lower heart rates in placebo

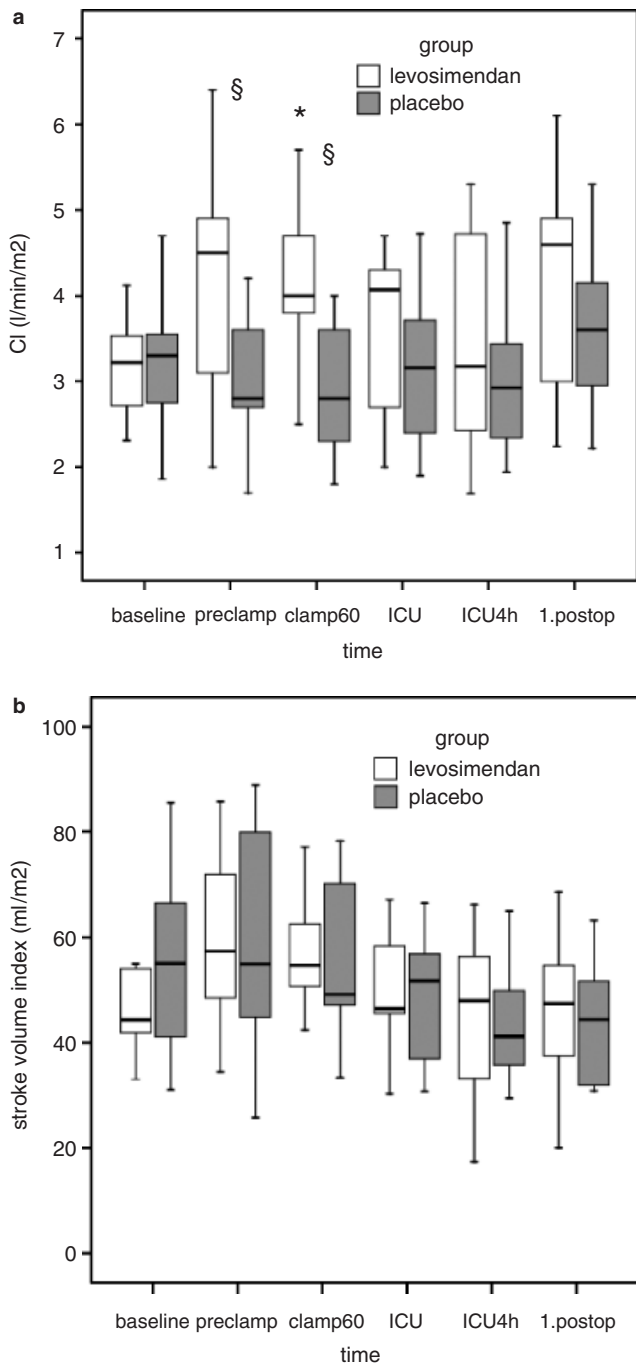


Fig.1. Cardiac index (a) and stroke volume index (b) in levosimendan-(white columns) and placebo-treated patients (black columns) at baseline, before aortic clamping (pre-clamp), 60 min after aortic clamping (clamp60), at admission to the intensive care unit (ICU), 4 h after admission (ICU4h) and in the first post-operative morning (1. postop). *P<0.05 by Wilcoxon's signed-rank test (against the baseline) and §P<0.05 by Mann-Whitney U-test (between the groups).

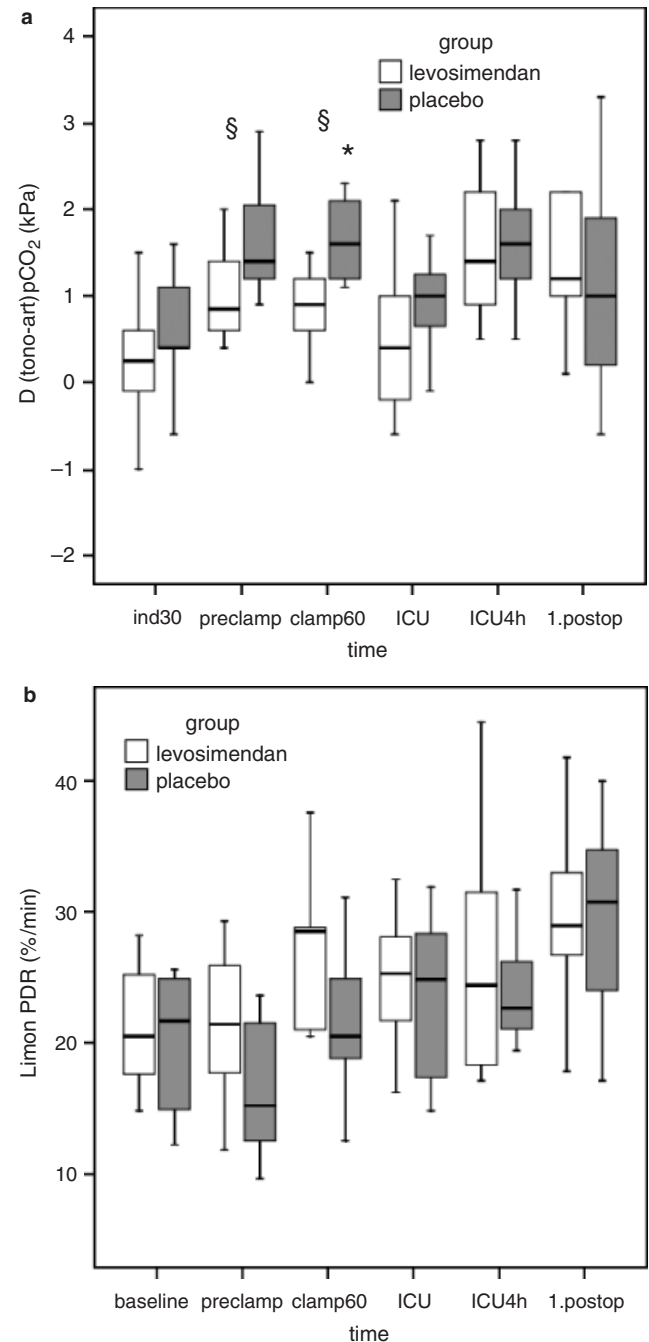


Fig.2. Gastric mucosal to arterial pCO₂ gradient (a) and indocyanine green disappearance rate (ICG-PDR)(b) in levosimendan (white columns) and placebo-treated groups (black columns) patients at the baseline or 30 min after the induction of anaesthesia (ind30) and before aortic clamping (pre-clamp), 60 min after aortic clamping (clamp60), at admission to intensive care unit (ICU), 4 h after admission (ICU4h) and in the first post-operative morning (1. postop). *P<0.05 by Wilcoxon's signed-rank test (against the baseline) and §P<0.05 by Mann-Whitney U-test (between the groups).

group in the beginning of surgery (Table 2). In the previous literature, levosimendan was associated with improved cardiac performance without increasing myocardial oxygen consumption (9). Even though we did not measure myocardial oxygen consumption, it is reasonable to speculate that stable heart rates may be associated with a higher myocardial oxygen consumption compared with our control patients. Previously, levosimendan was not associated with increased heart rate during septic shock-related myocardial depression (18). In our study, no troponin T release occurred in either of the groups. ST-segment changes were not detected during the study period. These findings may indicate that no myocardial ischaemia occurred and somewhat higher heart rates were well tolerated by our levosimendan-treated patients.

There are limitations in our present clinical study: Firstly, the number of patients included was small and the limited data available with PDR method do not make it possible to calculate the sample size reliably. However, we used previous data from studies on splanchnic circulation in similar clinical settings, which may have resulted in too a small sample size. The use for other vasoactive drugs was an additional confounding factor: the need for norepinephrine was different between the groups, and therefore interaction between levosimendan and norepinephrine in terms of mucosal perfusion is possible. From the clinical perspective, this is an important finding. The need of other vasoactive drugs may increase in association with the use of levosimendan. Thereby, the overall effects on different vascular beds may be unpredictable. It might be an overstatement to simply suggest that the results presented herein could only be related to levosimendan. Secondly, we could not estimate the total splanchnic blood flow directly (hepatic vein catheter). Rather we used the peripheral detection of ICG clearance. After injection into circulation, ICG is nearly completely eliminated unchanged by the liver into bile without enterohepatic recirculation (28). The ICG-PDR has been found to be a good predictor of survival in critically ill patients (29, 30). At best, it reflects the total splanchnic blood flow without separating hepatic arterial from portal flow (31). We did not measure gastric mucosal capillary perfusion (laser Doppler flow). Rather, we used a surrogate for the gastric perfusion. To some degree, we can state the gastric mucosal-arterial $p\text{CO}_2$ gradient reflects the adequacy of total splanchnic perfusion.

In conclusion, levosimendan, a new inodilator, increases the cardiac index, and seems not to favour total the splanchnic blood flow but may direct blood flow preferentially towards the gastric wall in abdominal aortic surgery.

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Address:
Heli Leppikangas
Department of Intensive Care
Tampere University Hospital
PO Box 2000
33521 Tampere
Finland
e-mail: heli.leppikangas@pshp.fi

CARDIOVASCULAR

Preoperative levosimendan infusion in combined aortic valve and coronary bypass surgery

H. Leppikangas^{1*}, K. Järvelä², T. Sisto², P. Maaranen², M. Virtanen², P. Lehto², S. Karlsson³, T. Kööbi⁴ and L. Lindgren¹

¹ Department of Anaesthesia, Tampere University Hospital, ² Heart Center, Tampere University Hospital, ³ Department of Intensive Care, Tampere University Hospital, and ⁴ Department of Clinical Physiology, Tampere University Hospital, PO Box 2000, Tampere 33521, Finland

* Corresponding author. E-mail: heli.leppikangas@pshp.fi

Editor's key points

- Levosimendan is an inodilator.
- The aim was to see whether this drug, given before operation, offered any benefits in high-risk cardiac surgery.
- After operation, patients in the levosimendan group maintained higher cardiac index and stroke volume than the control group.
- Levosimendan improved perioperative haemodynamics in patients undergoing high-risk cardiac surgery.

Background. Cardiopulmonary bypass may have detrimental effects on intestinal function and decrease the concentrations of the active, long-acting metabolites of levosimendan, an inodilator used to improve cardiac function. The aim of this study was to evaluate the haemodynamic effects of preoperative levosimendan in patients undergoing high-risk cardiac surgery.

Methods. Twenty-four patients were randomized to receive levosimendan (12 µg bolus followed by an infusion of 0.2 µg kg⁻¹ min⁻¹) or a placebo 24 h before surgery. The inclusion criteria were left ventricular ejection fraction (LVEF) <50% or LV hypertrophy indicated by a wall thickness of >12 mm. Haemodynamics were recorded every hour for 24 h (pulmonary artery catheter) and daily until postoperative day 4 (whole-body impedance cardiography). Doppler echocardiography with tissue Doppler imaging was used to assess systolic and diastolic cardiac function.

Results. The cardiac index (CI) and stroke volume index (SI) were higher in the levosimendan group (LG) for the 4 day postoperative period ($P < 0.05$); on the fourth postoperative day, the CI was 3.0 litre m⁻² min⁻¹ in the LG compared with 2.4 litre m⁻² min⁻¹ in the control group (CG) and the SI was 30 vs 25 ml m⁻², respectively. The LVEF measured at baseline and on the fourth postoperative morning decreased in the CG, but was maintained in the LG.

Conclusions. Levosimendan improved haemodynamics compared with a placebo in patients undergoing high-risk cardiac surgery. The concentrations of levosimendan's metabolites were higher compared with earlier studies using perioperative dosing.

URL: <https://register.clinicaltrials.gov>
ClinicalTrials.gov Identifier: NCT01210976

Keywords: heart, coronary artery bypass; heart, inotropism; surgery, cardiovascular

Accepted for publication: 3 December 2010

Patients undergoing aortic valve replacement (AVR) surgery combined with coronary artery bypass grafting (CABG) are at risk for left ventricular (LV) dysfunction.¹ Vasoactive therapy could be required for weaning from cardiopulmonary bypass (CPB) or to increase tissue perfusion in the perioperative period.² Maintaining adequate cardiovascular function is essential for sufficient oxygen delivery. Adequate oxygen delivery and normal mixed venous saturation (Sv_O₂) values during the immediate postoperative period after cardiac surgery can decrease morbidity and, therefore, reduce the length of the hospital stay.³ Levosimendan, a calcium-sensitizing inodilator, enhances myocardial contractility and causes both coronary and peripheral vasodilatation⁴ without increasing the myocardial oxygen demand.⁵ Levosimendan is used to improve cardiac function and was used in patients

suffering from congestive heart failure,⁶ before and after operation after CABG,^{7,8} and for patients in septic shock.⁹

Levosimendan has an intermediate metabolite OR-1855, which is further acetylated to the active metabolite OR-1896. It is formed by intestinal bacteria and its half-life is 77 (9) h.¹⁰ The metabolite OR-1896 has haemodynamic and pharmacological properties similar to the parent drug.^{11,12} CPB is associated with an imbalance in oxygen demand and supply in the hepatosplanchnic region,¹³ which may have detrimental effects on intestinal function. Patients infused with levosimendan during the perioperative period may lack the active metabolite. Our aim was to infuse levosimendan on the day before surgery to ensure the presence of OR-1896. Here, we describe the haemodynamic effects of levosimendan, compared with a placebo, in patients undergoing AVR together with CABG.

Methods

Twenty-four patients undergoing AVR with CABG were enrolled in the study. The inclusion criteria were LV ejection fraction (LVEF) <50% or LV hypertrophy, as indicated by a wall thickness of >12 mm. The exclusion criterion was a known allergy to levosimendan. The ethics committee of the hospital approved the study protocol and it was registered with EudraCT (ref: 2008-001672-70). Written informed consent was obtained from all patients before enrolling. The same surgical team performed all the operations (T.S., P.M., and K.J.).

The patients were randomized (sealed opaque envelopes) into two groups: the levosimendan group (LG) and the control group (CG). A study nurse prepared and diluted the study drugs, thereby ensuring that all personnel were blinded to the group assignment. Infusion of the study drug started the day before surgery. In the LG, patients received a levosimendan (Simdax®; Orion Pharma, Espoo, Finland) bolus 12 µg kg⁻¹ in 10 min followed by a 24 h infusion at a rate of 0.2 µg kg⁻¹ min⁻¹. In the CG, patients received a placebo bolus and infusion, which were made to look identical to levosimendan with water-soluble vitamin concentrate (Soluvit®; Fresenius Kabi, Uppsala, Sweden, 10 ml diluted in 500 ml of glucose 5%).

The anaesthesia and CPB were performed according to the hospital's clinical practice. A pulmonary artery catheter (Criticath SP5537; Becton Dickinson, Singapore) was inserted for haemodynamic monitoring in the operating theatre.

At admission to the operating theatre and thereafter, the goals and means of haemodynamic support were to keep the pulmonary capillary wedge pressure (PCWP) at the level of 12–18 mm Hg with fluid administration, the mean arterial pressure between 60 and 90 mm Hg with the aid of norepinephrine or sodium nitroprusside, and the cardiac index (CI) above 1.8 litre min⁻¹ m⁻² with dobutamine. If the CI was still <1.8 litre min⁻¹ m⁻², milrinone or epinephrine was added.

Haemodynamic measurements were made before and after induction of anaesthesia, after weaning from perfusion, after sternum closure, at admission to the intensive care unit (ICU), and hourly thereafter for 24 h. Blood gases and mixed venous saturation, lactate, and haematocrit were taken according to the same schedule, except at 4 h intervals in the ICU. Levosimendan, OR-1896, and OR-1855 concentrations were measured at baseline, before induction of anaesthesia, and at 72 and 96 h. N-terminal pro-B-type natriuretic peptide (NT-proBNP) was taken at baseline, before induction of anaesthesia, and on the fourth postoperative morning. P-creatin kinase (P-CK) and P-creatin kinase-MB subunit (P-CK-MB) were taken 6 h after operation and on the first and second postoperative mornings.

Whole-body impedance cardiography

CircMon B202 (JR Medical Ltd, Tallinn, Estonia) was used for the measurement of whole-body impedance cardiography (ICG_{WB})-derived stroke volume index (SI), heart rate (HR), CI, left cardiac work index (LCWI), systemic vascular

resistance index (SVRI), and extracellular water (ECW). This method is described in detail elsewhere.¹⁴ Disposable electrocardiogram electrodes (Blue sensor type R-00-S/25, Ambu®, Denmark) were used. A pair of electrically connected current electrodes was placed on distal parts of the extremities, immediately proximal to the wrists and ankles. Voltage electrodes were placed proximal to the current electrodes; the distance between the electrodes was 5 cm. Measurements were done in the supine position, and the patient's limbs were isolated from the trunk to prevent an electric connection during the bioimpedance measurements. ECW¹⁴ was calculated using the equation: $ECW = K \cdot H^2 / R$, where H was the patient's height (cm), R the resistive part of the whole-body bioimpedance (Ω), and K the correction factor ($K_{\text{males}} = 0.078$, $K_{\text{females}} = 0.095$). Arterial blood pressure was measured non-invasively using Accutorr 4 (DatascopeCorp, Montvale, NJ, USA).

After baseline measurements, the study drug infusion was started. On the morning of surgery, the ICG_{WB} measurements were done before the patients received their scheduled medications. Postoperative measurements were performed on the first and fourth postoperative mornings.

Echocardiography

Transthoracic echocardiography was performed by the same cardiologists (M.V. and P.L.) at baseline and on the first and fourth postoperative days. An Esaote Mylab 30CV (ESAOTE S.p.A, Firenze, Italy) was used for standard measurements. The LVEF was measured from the two-dimensional echocardiography using biplane Simpson's method. The M-mode was used to measure the LV mass and conventional cardiac dimensions. The mitral and aortic flow patterns were recorded with Doppler echocardiography and the mitral annular velocities with tissue Doppler imaging. Systolic pulmonary pressure was estimated non-invasively by adding the peak gradient of tricuspid regurgitation to the right atrial pressure estimated from the dimensions of the inferior vena cava and its decrease on deep inspiration.

Statistical methods

We calculated the sample size based on published data.¹⁵ Sample size was estimated as 10 patients per group, with a two-sided α level of 0.05 and a power of 0.80 to detect a 7.5 ml m⁻² difference in SI, with an SD of 5, at the end of study. We decided to enrol 12 patients in each group in the case of drop outs. Baseline variables were tested using Student's *t*-test and Fisher's exact test for continuous and categorical variables, respectively. Normally distributed variables were tested using the analysis of variance for repeated measures (*RANOVA*) model with effects for treatment, time, and treatment \times time interaction. The first measurement was used as a covariate in haemodynamic measurements. Time point-wise comparisons were done with the *T*-test. Cumulative doses of vasoactive medications were compared between groups using the Mann-Whitney *U*-test. Values are

presented as mean (SD). The analyses were carried out using SPSS for Windows, version 18.0 (SPSS Inc., Chicago, IL, USA).

Results

Patient characteristics and operative data are presented in Table 1. No significant differences were detected between the treatment groups. The SI, CI, and HR measured by ICG_{WB} are presented in Figure 1. LCWI was higher in the LG compared with the CG ($P=0.003$, RANOVA) and there was no difference in systolic or mean arterial pressure between the groups during the study period. There was a trend towards lower values for SVRI in the LG compared with the CG during the postoperative period ($P=NS$). At baseline, transmitral flow velocity combined with annular velocity (E/E') was elevated in both groups. E/E' is presented in Figure 2A. Left atrial volume indexes were also suggestive for diastolic dysfunction (39.4 ml m^{-2} in the CG and 39.9 ml m^{-2} in the LG).

The LVEFs were normal in both groups at baseline. In the postoperative period, the LVEF decreased in the CG, but was maintained in the LG (Fig. 2B). On the fourth postoperative morning, the gradient measured across the aortic valve (peak/mean) was bigger in the LG compared with the CG: [33 (8)/17 (4) mm Hg] vs [24 (7)/12 (4) mm Hg], respectively, $P<0.05$ between groups. There was no difference in aortic valve size or in the valve type (a mechanical or biological) between the groups.

Levosimendan was rapidly absorbed and eliminated from plasma after study drug infusion ended. Twenty-four h after the start of infusion, the mean concentration was $30 (20) \text{ ng ml}^{-1}$ and it was below the lower limit of quantification (i.e. $<0.200 \text{ ng ml}^{-1}$) at 48 h. Both metabolites had a growing

trend (Fig. 3) until 96 h after the start of the levosimendan infusion.

Fluid input was greater in the LG compared with the CG during drug infusion (from baseline to the morning of surgery) ($P=0.03$). From the morning of surgery to the first postoperative morning, there was no difference in total fluid balance $11\,631 (3291) \text{ ml}$ in the LG vs $9620 (2789) \text{ ml}$ in the CG ($P=0.09$). The

Table 1 Patient characteristics and operative data. Results are expressed as mean (SD) or numbers. ACE, angiotensin-converting enzyme; LVEF, left ventricular ejection fraction; CPB, cardiopulmonary bypass

	Placebo (n = 12)	Levosimendan (n = 12)
Sex (male)	8	10
Age (yr)	75 (8)	76 (10)
Weight (kg)	77 (12)	84 (13)
Body surface area (m ²)	1.88 (0.20)	1.97 (0.18)
Preoperative β -blocker	7	11
Preoperative ACE inhibitor	4	6
Preoperative Ca-channel blocker	2	5
Preoperative statin	11	9
Preoperative LVEF (%)	69 (9)	63 (9)
Preoperative aortic valve gradient (mm Hg) peak	78 (12)	89 (22)
Mean	47 (8)	56 (17)
Operation time (min)	220 (48)	240 (40)
CPB time (min)	125 (29)	133 (21)

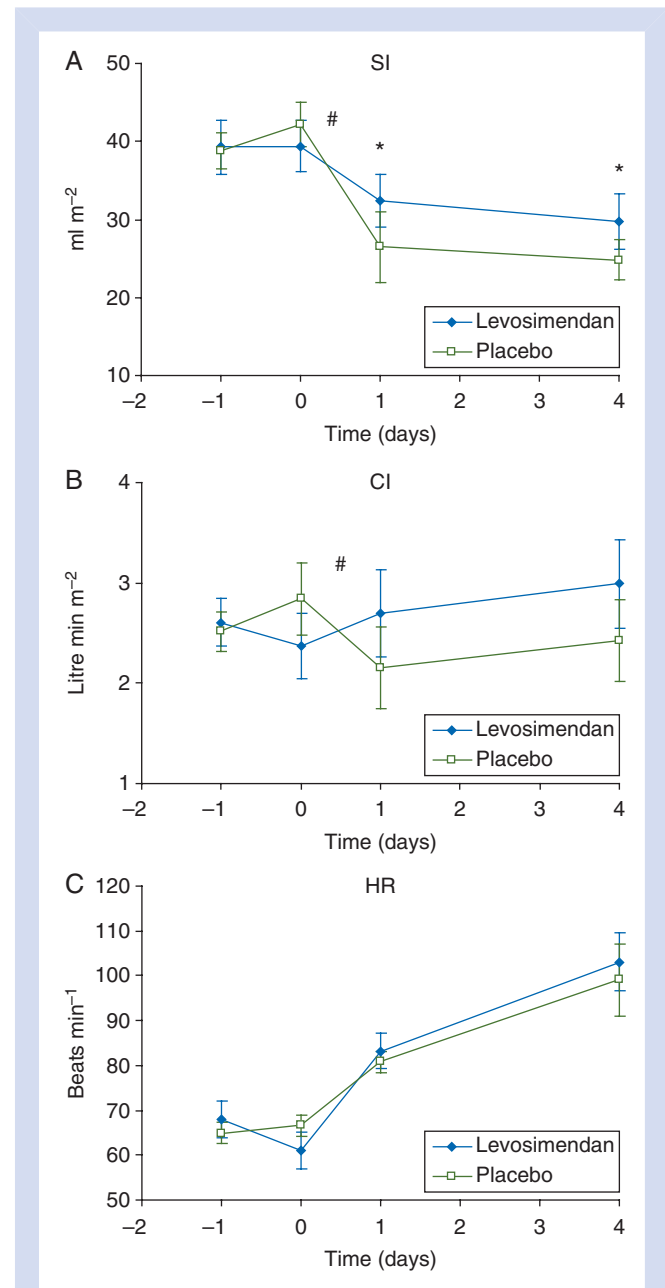


Fig 1 (A) SI, (B) CI, and (C) HR at baseline (-1), on the morning of surgery (0), and on the first (1) and fourth (4) postoperative mornings, measured by whole-body impedance cardiography. Results are expressed as mean (confidence intervals). * $P<0.05$ between the groups and # the significant difference between the groups from the first postoperative morning to the morning before surgery.

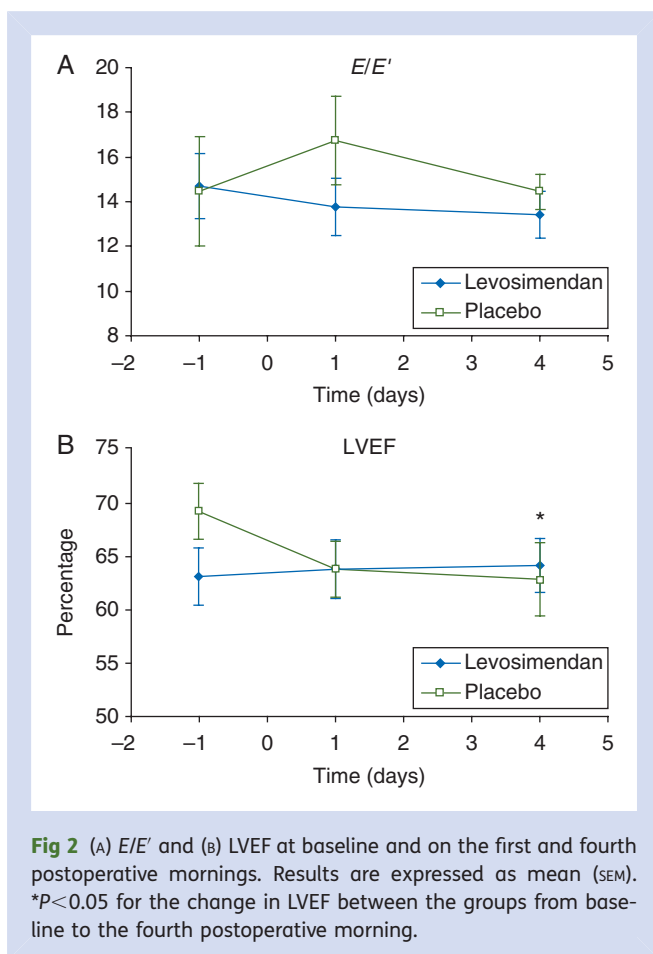


Fig 2 (A) E/E' and (B) LVEF at baseline and on the first and fourth postoperative mornings. Results are expressed as mean (SEM). * $P < 0.05$ for the change in LVEF between the groups from baseline to the fourth postoperative morning.

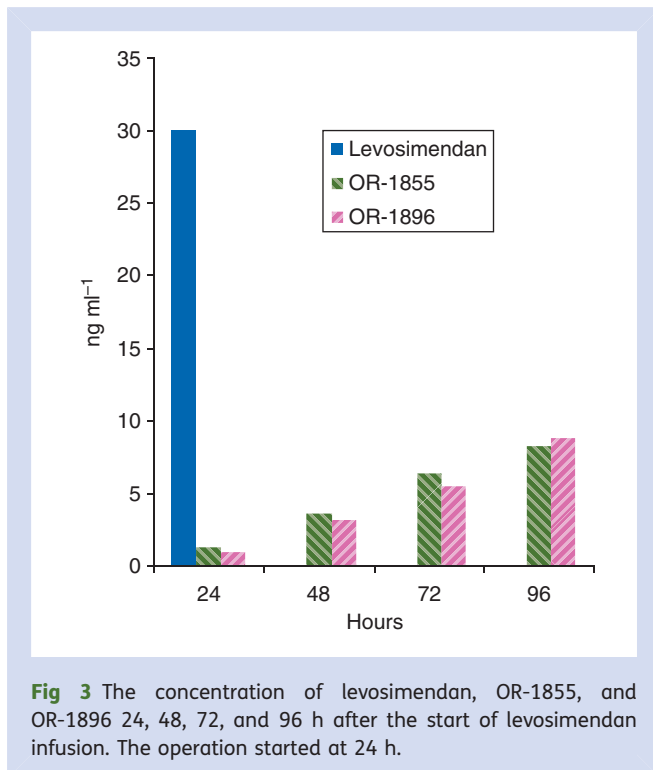


Fig 3 The concentration of levosimendan, OR-1855, and OR-1896 24, 48, 72, and 96 h after the start of levosimendan infusion. The operation started at 24 h.

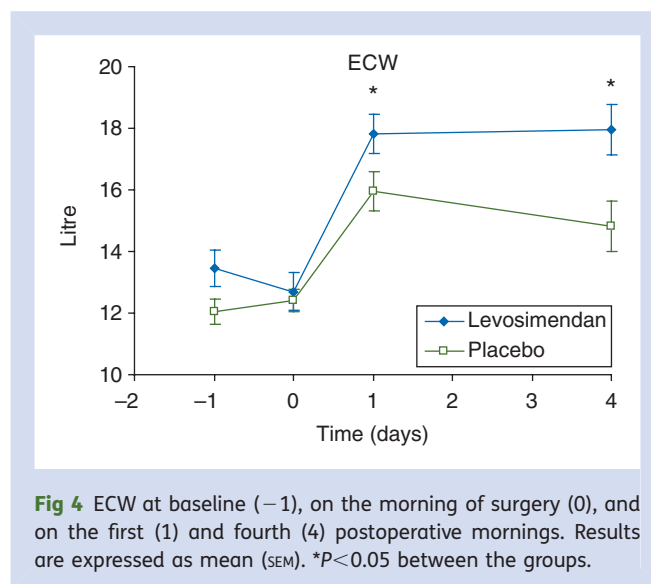


Fig 4 ECW at baseline (-1), on the morning of surgery (0), and on the first (1) and fourth (4) postoperative mornings. Results are expressed as mean (SEM). * $P < 0.05$ between the groups.

fluid input (crystalloids, colloids, and blood products) and output was comparable between the groups. ECW was comparable between the groups before operation, but it was significantly higher in the LG, compared with the CG, during the postoperative period ($P = 0.008$ RANOVA; Fig. 4).

The LG required more norepinephrine during surgery and in the ICU compared with the CG [1.87 (1.23) vs 0.37 (0.57) mg, $P = 0.001$ and 1.22 (0.99) vs 0.18 (0.30) mg, $P = 0.001$, respectively]. Norepinephrine infusion was discontinued in all patients by the first postoperative morning. Otherwise, the need for vasoactive medication did not differ between the groups. At baseline, NT-proBNP was elevated in 10 patients in the LG compared with eight patients in the CG; 2088 (2541) vs 1232 (1010) ng litre⁻¹, $P = 0.29$, respectively. After operation, NT-proBNP increased in both groups on the fourth postoperative morning; 3329 (2662) ng litre⁻¹ in the LG vs 3298 (1933) ng litre⁻¹ in the CG ($P = \text{NS}$). P-CK-MB and systemic lactate values were comparable between the groups throughout the study period. Myocardial injury was described using a strict criterion of P-CK-MB. In two patients in both groups, P-CK-MB exceeded 75 U litre⁻¹ in the first postoperative morning. By the second postoperative morning, P-CK-MB was above 75 U litre⁻¹ in one patient in the LG. The mechanical ventilation time did not differ between the groups: 586 (615) min in the LG vs. 340 (88) min in the CG ($P = 0.20$). The length of stay in the ICU was 25.3 (9.7) h in the LG vs 25.6 (10.1) h in the CG and in the hospital 8.6 (3.3) days in the LG vs 8.8 (5.6) days in the CG ($P = \text{NS}$). One patient in the LG died on the first postoperative morning. The death was not related to study drug infusion.

Discussion

In this prospective randomized study, levosimendan improved haemodynamics during the 4 day postoperative period, when infused a day before surgery. The formation of metabolites was documented for this 4 day postoperative period.

Peak concentrations of the levosimendan metabolites have been observed at 2–4 days in heart failure patients,¹⁶ whereas the concentration peaked at day 6 in cardiac surgery patients studied by Eriksson and colleagues.¹⁷ In our study, the concentrations of metabolites showed an increasing trend until day 4. The concentrations on day 4 were higher than the peak concentration in the study by Eriksson and colleagues; the mean concentration of OR-1855 was 8.4 (3.3) ng ml⁻¹ compared with the peak value of 6.6 (4.9) ng ml⁻¹ reported by Eriksson and colleagues on day 6. Similarly, the mean concentration of OR-1896 was 8.8 (4.9) ng ml⁻¹ compared with 7.7 (6.3) ng ml⁻¹. This supports our hypothesis that the formation of the metabolites is not disturbed by possible hypoperfusion caused by CPB, when levosimendan is started before operation. Eriksson and colleagues¹⁷ also showed the concentration of levosimendan peaked ~2 h after infusion began. In our study, patients underwent surgery 24 h after starting the study drug infusion. The patients received the beneficial effects of levosimendan before operation and the metabolites were starting to increase early in the postoperative phase. The lowest concentration of OR-1896 that enhances haemodynamics is not available.

Levosimendan augments cardiac performance after cardiac surgery with CPB in patients with normal preoperative LV function.⁸ In the present study, the LVEF was within the normal range in both groups at baseline. The LVEF decreased from the baseline value on the first and fourth postoperative days in the CG, but it remained constant in the levosimendan-treated patients. The effects of levosimendan, measured by ICG_{WB}, lasted throughout the study period. The SI remained significantly better in the LG compared with the CG and there was no difference in HR between the groups; therefore, the higher CI values in the LG were due to enhanced SI rather than tachycardia. Levosimendan was able to support cardiac function, which decreased in the CG despite the normal systolic function at baseline. The duration of the increase in cardiac output was 12–13 days in congestive heart failure patients.¹⁶ This is consistent with our finding, although we do not know how long the difference remained in our study population. These observations suggest that levosimendan may prevent the myocardial dysfunction that often occurs after cardiac surgery.¹⁸ The authors expected a decrease in the requirements of other inotropes; however, no differences were found in the requirements for inotropic support between the two study groups.

On the fourth postoperative morning, the gradient measured across the aortic valve was higher in the LG compared with the CG. The implanted valves were comparable in both groups. Higher values in the LG were likely due to the increased SI. Diastolic dysfunction was suggested by Doppler and tissue Doppler imaging. The diastolic function was assessed with *E/E'*, which combines the influence of transmitral driving pressure and myocardial relaxation. De Luca and colleagues¹⁹ have shown a decrease in LV filling pressure, assessed by *E/E'*, after levosimendan treatment.

In the present study, the difference between the groups was not significant despite a decreasing trend in the LG and an increasing trend in the CG. This may be due to the haemodynamic treatment protocol, which aimed at standardized filling pressures. The LG needed more fluids to achieve pre-set filling pressure. Statistically significant changes were not seen in any other measurements concerning diastolic function. The left atrial volume index, peak Doppler velocities of early (*E*) and late diastolic (*A*) flow, deceleration time of *E*-wave, and *E/A* ratio were not significantly different between treatment groups at any time point. Still, the SI increased, the LVEF remained constant, and diastolic function assessed with *E/E'* showed an increasing trend in the LG compared with the CG.

There was no significant difference in the NT-proBNP or P-CK-MB between the groups. This is in the NT-proBNP probably because, in our treatment protocol, fluids were infused to meet the PCWP goal; therefore, we were unable to see the decrease in filling pressures. A recent meta-analysis suggests that levosimendan is associated with a reduction in cardiac troponin release in cardiac surgery patients.²⁰ The release of the injury marker we used did not confirm this finding.

Tasouli and colleagues investigated starting the levosimendan infusion at different time points. In their study, patients were randomized to receive a continuous infusion of levosimendan (0.1 µg kg⁻¹ min⁻¹) started either intraoperatively or after operation in the ICU. The earlier start was associated with a shorter ICU and hospital stay.²¹ In our study, the length of the ICU or hospital stay did not differ between the groups, which may be due to small sample size.

ICG_{WB} is reportedly a reliable method of measuring cardiac output; comparisons with a bolus and continuous thermodilution and direct Fick methods showed that ICG_{WB} measures cardiac output accurately in different conditions (in the supine position, during head-up tilt, after induction of anaesthesia, and CABG).^{14 22} Differences in cardiac output values between the ICG_{WB} and thermodilution methods were comparable with those between direct Fick and thermodilution methods. The repeatability of ICG_{WB} was nearly twice as good as that of thermodilution.^{23 24} Therefore, ICG_{WB} is an adequate method to estimate cardiac output and its changes.

The exact volume of ECW in man is unknown. In invasive diagnostics, the distribution space of substances used for ECW volume estimation ranges from 15% (inulin and mannitol) to 23% (ions such as bromide and chloride) of body weight.²⁵ The ICG_{WB} device calculates ECW volume on the basis of the equation derived by Kolesnikov and colleagues,²⁶ which is fitted to thiosulphate space giving ECW values around 16% of body weight. The decrease in systemic vascular resistance caused by levosimendan resulted in changes in the fluid distribution from the central to peripheral vasculature. We do not believe this would significantly influence the measured *R* in the equation, and thereby increase the calculated ECW. The higher ECW values measured after

operation in the LG were probably due to the increased need for fluids in the preoperative phase.

Levosimendan may exert cardioprotective effects by the activation of K_{ATP} channels.^{27 28} The authors made an effort to minimize confounding factors by selecting total i.v. anaesthesia. Volatile anaesthetics are known to affect K_{ATP} channel opening on reperfusion.²⁹ We also tried to minimize other confounding factors; two surgeons performed all the operations and one anaesthesiologist was responsible for the anaesthesia and CPB. The elective patients received the levosimendan bolus under continuous haemodynamic monitoring. In order to maintain adequate blood pressure, the patients in the LG needed volume loading. None of the patients needed norepinephrine. We suggest that in this patient group, the bolus is given with caution.

A limitation of this study is the small sample size. The sample size was calculated based on differences in stroke index. The study was not designed and powered to detect differences in other variables, for example, diastolic function or hospital stay. Another limitation in the present study is that the metabolites of levosimendan were only measured for 4 days and the peak concentrations were probably not seen. The cardiac surgery patients usually are transferred from our hospital to other hospitals on the fourth or fifth postoperative day; therefore, we were not able to take samples after the fourth day. The patients were invited to the hospital 3 h earlier than they normally would have been. If this can be done in the clinical practice, the preoperative infusion of levosimendan is not adding costs beyond the price of the drug.

In conclusion, the present study shows that CI and SI are enhanced for four postoperative days when levosimendan is infused before operation. The formation of levosimendan's metabolites also seems more efficient compared with perioperative dosing.

Acknowledgements

We thank Minna-Liisa Peltola and Pirjo Järventausta, research nurses, for their valuable technical assistance. Heini Huhtala, MS, was consulted for the statistical methods used.

Conflict of interest

H.L. and L.L. have lectured for Orion, the manufacturer.

Funding

This work was supported by grants from the Finnish Cultural Foundation, Competitive Research Funding of the Pirkanmaa Hospital District, Tampere University Hospital, Ida Montin Foundation, and Orion Pharma Ltd.

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ORIGINAL RESEARCH

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Effect of levosimendan in experimental verapamil-induced myocardial depression

Jouni Kurola*¹, Heli Leppikangas², Jarkko Magga¹, Leena Lindgren², Vesa Kiviniemi³, Juha Rutanen¹ and Esko Ruokonen¹

Abstract

Background: Calcium antagonist overdose can cause severe deterioration of hemodynamics unresponsive to treatment with beta adrenergic inotropes. The aim of the study was to evaluate in an experimental model the effects of levosimendan during severe calcium antagonist intoxication.

Methods: Twelve landrace-pigs were intoxicated with intravenous verapamil at escalating infusion rates. The infusion containing 2.5 mg/ml verapamil was used aiming to a reduction of cardiac output by 40% from the baseline value. Intoxicated pigs were randomized into two groups: control (saline) and levosimendan (intravenous bolus). Inotropic effect was measured as a change in a maximum of the positive slope of the left ventricular pressure (LV dP/dt). The survival and hemodynamics of the animals were followed for 120 min after the targeted reduction of cardiac output.

Results: In the control group, five out of six pigs died during the experiment. In the levosimendan group, one pig died before completion of the experiment ($p = 0.04$). In the levosimendan group a change in LV dP/dt was positive in four out of six pigs compared to one out of six pigs in the control group ($p = ns$).

Conclusions: In this experimental model, the use of levosimendan was associated with improved survival.

Background

In the year 2004 more than 10000 toxic exposures to calcium channels blockers were reported in the United States. Of those exposures, 3.3% were associated with severe bradycardia, hypotension and acute negative inotropy. Altogether, there were 62 (0.6%) deaths due to calcium channel blocker overdoses. Verapamil and diltiazem involved the majority of these fatal poisonings [1]. The majority of the exposures occurred accidentally (79%), but a significant (18%) part was suicide attempts. Moreover, a small amount of overdoses was in children or due to iatrogenic treatments [1]. The number of toxic incidents is increasing [2,3].

Calcium channel blocker overdose causes intractable hypotension, bradycardia, cardiac conduction abnormalities and depression of myocardial contractility, leading to central nervous system (e.g. syncope, seizures and coma), respiratory (non-cardiogenic pulmonary edema) and metabolic (e.g. hyperglycemia and acidosis) disorders [4]. The management of calcium channel blocker poisoning

includes the use of a wide variety of medications and also non-pharmacological techniques [4]. The aims are to support vital functions and, on the other hand, to prevent the further absorption of calcium channel blockers from the gut with lavage and activated charcoal. The management of the cardiovascular symptoms is focused on normalization of sinus rate by atropine as well as pacing and restoration of normal arterial pressure (plasma volume expanders and catecholamines). The negative inotropy can be partly reversed by using β -adrenergic agonists, phosphodiesterase inhibitors, glucagon, insulin with dextrose and calcium salts [5]. Also a case report regarding the use of levosimendan has been published [6].

Both verapamil and diltiazem decrease myocardial contractility [7] at high plasma concentrations, as seen in acute poisoning [8]. The negative inotropy caused by these drugs is due to a direct cardiac effect, shown in vitro in Langendorff perfused isolated hearts [9]. The sustained effect of verapamil may be related to its active hepatic metabolite, nor-verapamil, which has 50% of the potency of the parent compound [10]. The symptoms of calcium channel blocker overdose do not always respond

* Correspondence: jouni.kurola@kuh.fi

¹ Department of Intensive Care, Kuopio University Hospital, Kuopio, Finland

to treatment with conventional beta adrenergic drugs. A rather new calcium sensitizer, levosimendan, is targeted to treatment of acute decompensated heart failure. Levosimendan induces a positive inotropic effect mediated through calcium-dependent binding of the compound to troponin C [11,12]. This mechanism of action increases sensitivity of contractile proteins for calcium. Levosimendan works also under extreme conditions e.g. acidosis [13] and sepsis [14]. Levosimendan also causes coronary dilation and systemic vasodilatation [15] through opening of ATP-sensitive potassium channels [16].

The aim of our study was to assess the effects of levosimendan in experimental porcine poisoning model of severe verapamil intoxication.

Methods

National Animal Ethics Committee of Finland approved the method. The animal care, welfare and procedures were carried out in accordance with the regulations of the Council of Europe.

Animals and anesthesia

Twelve [12] landrace-pigs (28 ± 5 kg) were deprived of food, but not water 12 h before the experiments. Premedication with medetomidine 50 μ g/kg, ketamine 10 mg/kg and fentanyl 5 μ g/kg intramuscularly was followed by cannulation of an ear vein and intravenous administration of 2 mg/kg of propofol for tracheotomy. Anesthesia was maintained with propofol (10 mg/kg/hour) and fentanyl (30 μ g/kg/hour). The animals were ventilated with a volume-controlled mode (Servo 900, Siemens, Elema AB, Solna Sweden) with 5 cmH₂O of positive end-expiratory pressure (PEEP). FIO₂ (0.3-0.6) was adjusted to keep PaO₂ levels between 13.3 kPa to 20 kPa. Tidal volume was kept at 10 ml/kg, and the minute ventilation was adjusted to maintain PaCO₂ levels between 4.5 to 5.5 kPa.

Animal preparation

A fluid-filled catheter was inserted into the right femoral artery (single-lumen central venous catheter, Arrow, Arrow International Inc, Reading, PA) and a pulmonary artery catheter (7.5F flow-directed, Arrow, Arrow International Inc, Reading, PA) introduced via the right internal jugular vein. The angiography (Impulse™, Boston Scientific, USA) catheter was inserted into left ventricle via left femoral artery to measure a change in a maximum of the positive slope of the left ventricular pressure (LVdP/dt). During instrumentation, the animals received 5 ml/kg/h infusions of 0.9% saline and gelatin (Gelo-fusine®, B. Braun Medical, Germany). Additional fluid was administered if necessary to keep pulmonary artery occlusion pressure (PAOP) between 5 and 8 mmHg. Body temperature of the animals was kept above 38°C using an operating table heater and warmed fluids.

Experimental protocol

After instrumentation, a stabilization period of at least 30 minutes was allowed followed by the baseline measurements. Verapamil intoxication was then induced by a long-lasting intravenous infusion containing 2.5 mg/ml of verapamil at an escalating rate into the right internal jugular vein. The rate of verapamil infusion was increased by 2.5 ml/h in every 15 minutes. The infusion was targeted to decrease cardiac output 40% from the baseline value. The administration and the amount of verapamil were based on a pilot trial in three pigs.

At completion of the verapamil intoxication phase, both the control and the levosimendan groups received a continuous infusion of verapamil 12.5 mg/h to maintain the toxicity level. Thereafter, animals in the control group received 25 ml bolus of saline in 15 minutes and the levosimendan group 1.25 mg levosimendan (Simdax®, Orion Pharma, Espoo, Finland) in the same volume and time. Arterial blood samples were obtained in heparinized tubes at Intox 0 and in the end of experiment for measurement of plasma concentrations of verapamil and nor-verapamil, calcium, lactate, sodium, potassium and glucose. The analytical method used was liquid chromatography-mass spectrometry. At the end of experiment, the surviving animals were killed with a high dose of verapamil. The total dose of verapamil given to the pigs was recorded at the end of the experiment.

Hemodynamic monitoring

Left ventricular pressure (LVP), mean arterial pressure (MAP), central venous pressure (CVP), end diastolic pressure (EDP) and pulmonary artery occlusion pressure (PAOP) were recorded with quartz pressure transducers and displayed continuously on a multimodular monitor (S/5 Compact Critical Care Monitor, Datex-Ohmeda™, Helsinki, Finland). All pressure transducers were calibrated simultaneously and zeroed to the level of the heart. The inotropic effect was measured as a change in a maximum of the positive slope of the left ventricular pressure (LV dP/dt). LV dP/dt was measured once a minute which represent a mean value over one minute cardiac cycles. A mean value of 5 minutes was recorded and its coefficient of variation in the control group was 3.6% (2.2; 5.8) and 4.1% (1.8; 10.5) in the levosimendan group (ns). Cardiac output (CO) was measured by a thermodilution technique and mean value of three measurements was used with room temperature saline injectates of 5 ml. (Datex-Ohmeda™, Helsinki, Finland). Heart rate (HR) was measured from the continuously monitored ECG.

Statistical analysis

Mann-Whitney test was used to analyze differences in hemodynamic and laboratory measures at preintoxication (baseline) and postintoxication (from Intox 0 to

Table 1: Comparison of hemodynamic values (median, IQR) between groups before verapamil infusion (Baseline) and at the time when intoxication was complete (Intox 0) (p = ns between levosimendan and control groups in both baseline and intoxic 0).

	Baseline	Intox 0
MAP		
control	95 (75;103)	43 (39;50)
levosimendan	100 (93;106)	44 (44;55)
CO		
control	4.6 (3.4;5.5)	2.4 (1.9;2.8)
levosimendan	4.4 (4.1;4.7)	2.1 (2.0;2.2)
LV dP/dt (mmHg/s)		
control	1730 (1548;1901)	761 (609;778)
levosimendan	2096 (2014;2238)	624 (518;736)
HR (beats/min)		
control	111 (98;132)	84 (78;94)
levosimendan	103 (95;111)	85 (72;93)
CVP (mmHg)		
control	6 (5;6)	7 (7;8)
levosimendan	5 (4;7)	7 (5;8)
EDP (mmHg)		
control	19 (12;23)	13 (11;15)
levosimendan	15 (13;16)	15 (12;16)

Intox 120) phases. Values are presented as median and interquartile range. Statistical analyses were done using a statistical program SPSS for Windows version 14.0 (SPSS® Inc. Chicago, USA). P-values of less than 0.05 were considered statistically significant.

Results

Baseline data on hemodynamics and the laboratory values of calcium and lactate are presented in Tables 1 and 2. There was no difference between the groups in baseline data excluding hemoglobin, which was higher in the levo-

Table 2: Comparison of calcium and lactate values (median, IQR) between groups before verapamil infusion (Baseline), at the time when intoxication was complete (Intox 0) and right before clinically estimated collapse of hemodynamics (End of experiment) ($p > 0.05$ between the groups).

	Baseline	Intox 0	End of experiment
Ca (mmol/l)			
control	1.20 (0.98;1.30)	1.10 (1.01;1.38)	1.06 (0.85;1.21)
levosimendan	1.02 (0.96;1.27)	1.00 (0.89;1.11)	0.98 (0.84;1.15)
Lactate (mmol/l)			
control	0.8 (0.6;0.7)	1.9 (1.1;1.9)	6.6 (4.7;8.7)
levosimendan	0.6 (0.6;0.7)	1.1 (0.9;1.4)	6.7 (1.2;8.6)

simendan group 73 (68; 73) g/l vs. 65 (66; 73) g/l ($p = 0.04$).

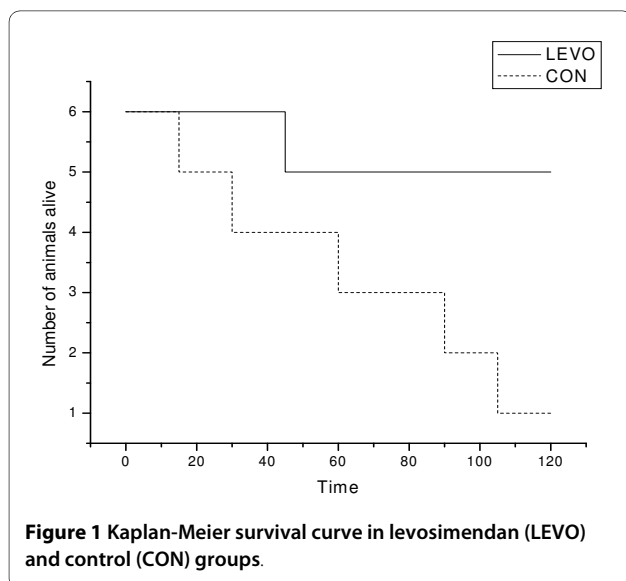
In each animal, cardiac output decreased by 40% as planned. In the planned control group the reduction was 45% (43; 54) and in the planned levosimendan group 49% (44; 50) ($p = ns$). The dose of verapamil required to induce toxicity was 22 (22; 37) mg and it took 53 (45; 71) minutes in the future control group and 22 (16; 29) mg and 53 (34; 60) minutes in the future levosimendan group ($p = ns$). Total amount of verapamil infused for both intoxication and maintenance period was 42 (38; 46) mg in the control group and 47 (46; 50) mg in levosimendan group ($p = ns$). Plasma concentrations of verapamil and

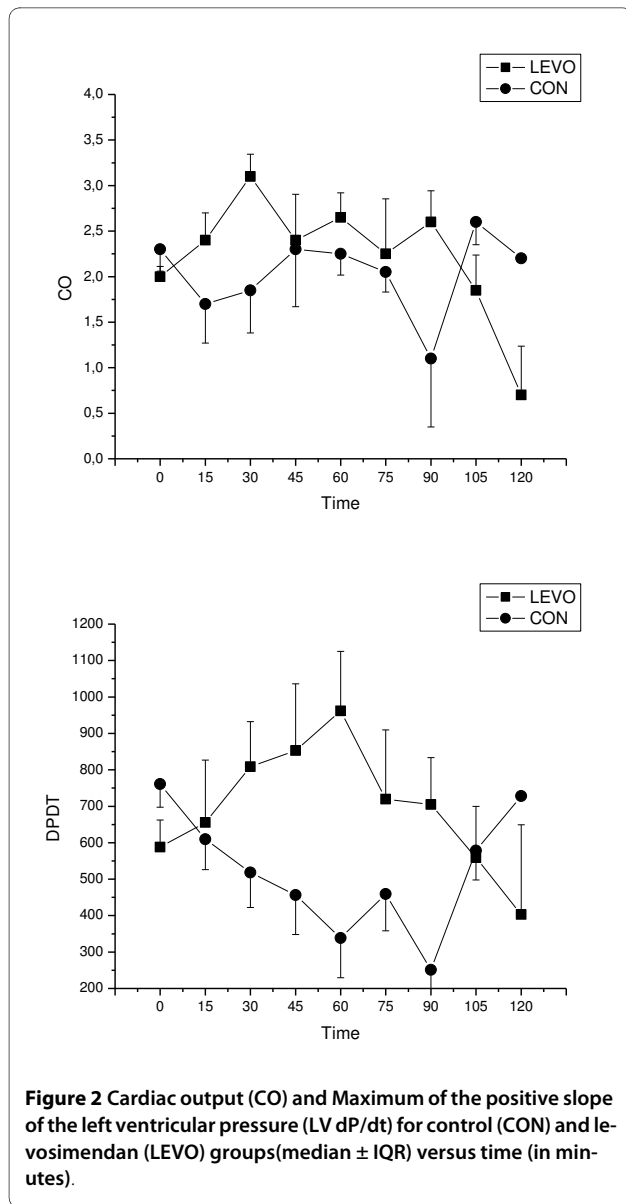
nor-verapamil at Intox 0 were in the levosimendan group 238.0 (222.0; 385.0) ng/ml and 3.0 (2.5; 8.1) ng/ml compared to the control group 293.5 (217.5; 365.5) ng/ml and 5.1 (2.2; 8.4) ng/ml ($p = ns$), respectively. There were no differences between levosimendan and control groups in verapamil and nor-verapamil concentrations between groups at the end of experiment 279.5(226.0; 315.0) ng/ml and 11.3 (10.4; 13.7) ng/ml vs. 300.0 (223.0; 339.8) ng/ml and 9.6 (9.8; 14.3) ng/ml ($p = ns$), respectively.

The hemodynamic and the laboratory data of lactate and calcium at the time point when intoxication was complete (Intox 0) are presented in Tables 1 and 2, and there were no differences between groups. There were no differences between groups in laboratory values at the end of the experiment ($p = ns$). The laboratory values of sodium, potassium and glucose were comparable between groups throughout the experiment ($p = ns$).

Five out of six pigs died during the experiment in the control group. In the levosimendan group one pig died before completion of the experiment. The median time alive from the completion of intoxication was 75 (60; 101) minutes in the control group and 120 (120; 120) minutes in the levosimendan group, respectively. The Kaplan-Meier survival curve is presented in Figure 1 ($p = 0.04$).

After completion of intoxication, the group receiving levosimendan had a tendency towards higher LV dP/dt than the control group, however there were no statistically significant differences either in LV dP/dt, CO, HR, MAP, CVP and EDP between groups (Figure 2, 3 and 4).

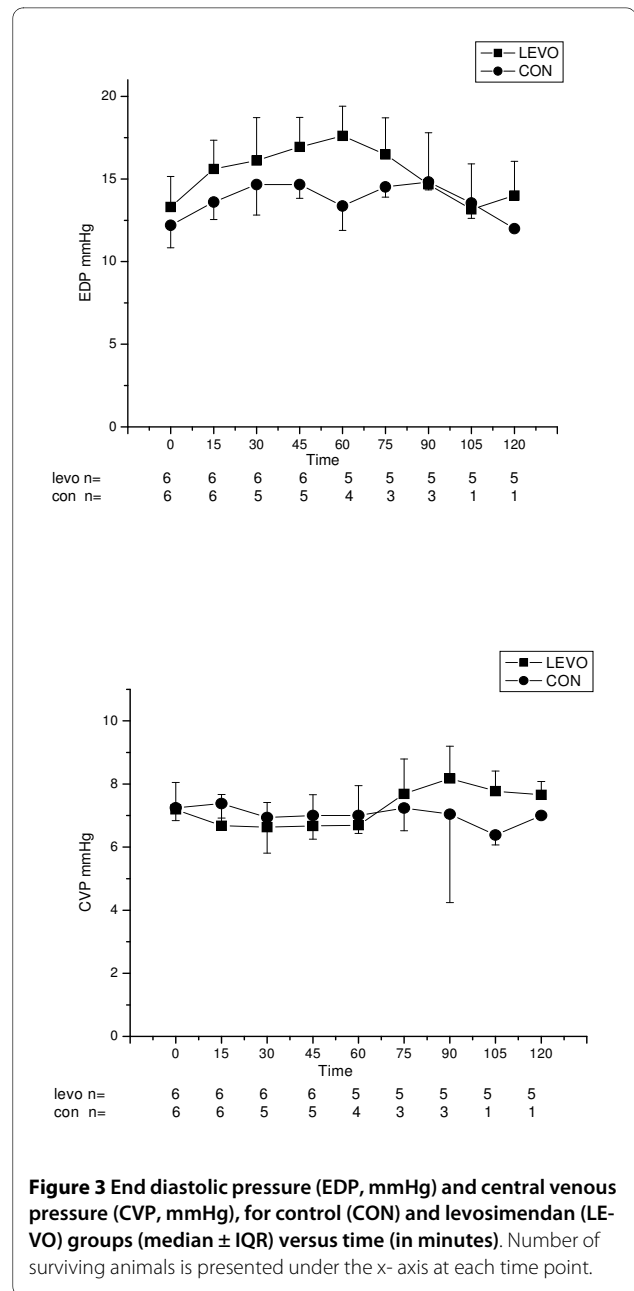




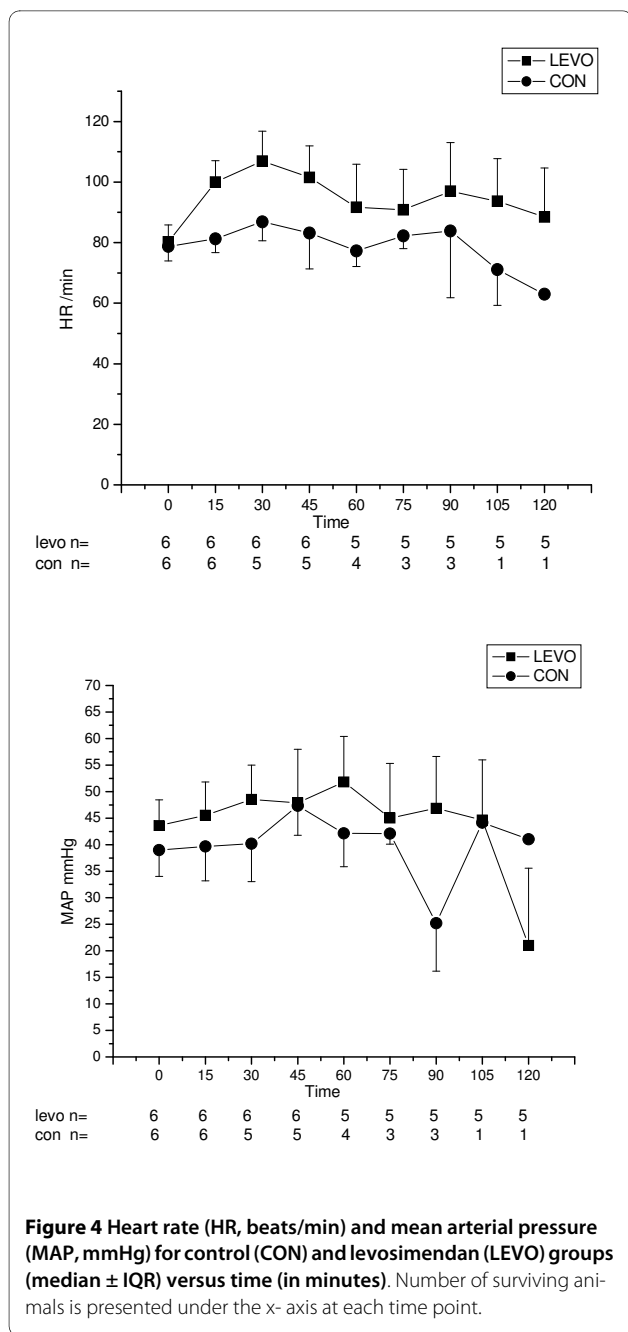
Discussion

The main finding of our study was that levosimendan improved survival in severe verapamil intoxication. In our experimental model, verapamil resulted in negative inotropy in control group assessed with dPdT. In contrast, heart rate was less prominently affected.

Verapamil intoxication is related to its intended action on myocardial and smooth muscle cells, where it competitively blocks cell surface slow calcium channels. Inhibition of calcium influx is responsible for depression of contractility causing a myocardial stunning-like syndrome [17-20]. The function and mechanical efficiency of stunned myocardium is depressed due to decreased sensitivity of the myofibrils to calcium [18].



Levosimendan enhances cardiac contraction by improving the use of available calcium rather by inundating the cell with excessive calcium [21]. The use of traditional inotropes is associated with increased energy consumption and arrhythmogenesis due to elevated intracellular calcium concentration leading to apoptosis in long term use [22,23]. Levosimendan causes vasodilatation via opening of adenosine triphosphatase-sensitive K⁺ channels [24]. This effect may contribute to coronary [25] and systemic [26] vasodilatation with the intravenous administration of levosimendan.



The levosimendan bolus was well tolerated. Even though the vasodilating effect of levosimendan has been well documented [26-28] it is noteworthy that it did not have deleterious effect on mean arterial pressure. It is conceivable that the inotropic effect of levosimendan was more prominent than the vasodilating effect.

Inotropic effect was measured as a change in a maximum of the positive slope of the left ventricular pressure (LV dP/dt). In the levosimendan group LV dP/dt increased by 38% from the baseline to the Intox 60 minutes, whereas LV dP/dt decreased in the control group by

31% during the same time interval. The same trend was seen for CO, but due to the small number of surviving animals in the control group, a significant difference was not reached between study groups. There were no clinical or statistical differences in HR, MAP, CVP and EDP between the study groups.

The first limitation of the study is that an animal model is not exactly like the toxicity seen in human beings. We chose a pig model because it has been used in previous studies of verapamil toxicity [29], and pigs have similar cardiovascular systems as humans [30]. The second limitation is the small number of animals and the survival rate was very low in control group; therefore detailed statistical analysis of hemodynamic differences between the groups was not possible. The third limitation is the use of intravenous verapamil as a substitute for oral ingestion that can prolong the absorption of verapamil. This limitation was minimized by continuing the verapamil infusion throughout the study to mimic oral ingestion. On the other hand, concentrations of verapamil and its active metabolite, nor-verapamil, were about similar in the two study groups. Although oral ingestion might have a different pharmacokinetics, according to hemodynamic data, we induced a severe verapamil poisoning.

In summary, treatment with levosimendan improved survival in pigs severely poisoned with verapamil. Levosimendan seemed to maintain cardiac performance especially during the early phase of intoxication without excessive vasodilatation. Confirmation of the effectiveness of levosimendan for pharmacotherapy of verapamil intoxication in humans requires further experiments.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

JK and HL participated to the design of the study, performed the study and prepared the manuscript. ER designed the study and prepared the manuscript. JR performed the study. JM and LL participated to the design of the study and VK made statistical analysis. All authors read and approved the final manuscript.

Author Details

¹Department of Intensive Care, Kuopio University Hospital, Kuopio, Finland, ²Department of Anesthesiology, Tampere University Hospital, Tampere, Finland and ³IT Centre, Kuopio University, Kuopio, Finland

Received: 26 October 2009 Accepted: 11 March 2010

Published: 11 March 2010

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- doi: 10.1186/1757-7241-18-12
- Cite this article as:** Kurola et al., Effect of levosimendan in experimental verapamil-induced myocardial depression *Scandinavian Journal of Trauma, Resuscitation and Emergency Medicine* 2010, **18**:12

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Levosimendan as a Rescue Drug in Experimental Propranolol-Induced Myocardial Depression: A Randomized Study

Heli Leppikangas, MD
 Esko Ruokonen, MD, PhD
 Juha Rutanen, MD, PhD
 Vesa Kiviniemi, PhLic
 Leena Lindgren, MD, PhD
 Jouni Kurola, MD, PhD

From the Department of Intensive Care, Kuopio University Hospital, Kuopio, Finland (Ruokonen, Rutanen, Kurola); the Department of Anaesthesia, Tampere University Hospital, Tampere, Finland (Leppikangas, Lindgren); and the IT Centre, University of Kuopio, Kuopio, Finland (Kiviniemi).

Study objective: Severe β -blocker intoxication remains a clinical challenge despite a variety of treatment options. Because of its unique mechanism of action, the new calcium sensitizer levosimendan may provide more prominent cardiac support compared with current medications used to reverse negative inotropy. We hypothesize that levosimendan could reverse propranolol-induced severe negative inotropy in a porcine model of β -blocker intoxication.

Methods: Twenty-four pigs were anesthetized and monitored. After severe propranolol intoxication was completed, animals were randomized into 3 groups. With a double-blind procedure, 9 animals received a 1.25-mg levosimendan bolus, followed by saline solution infusion, 9 animals received mean arterial pressure-targeted dobutamine infusion after saline solution bolus, and 6 animals received a saline solution bolus followed by saline solution infusion. Hemodynamic and laboratory data were collected during a follow-up period of 120 minutes.

Results: All 9 pigs in the levosimendan group survived. In contrast, 4 of 6 (67%) and 7 of 9 (78%) pigs died during the experiment in the placebo and the dobutamine groups, respectively. The levosimendan group showed improved change in the maximum positive slope of the left ventricular pressure, cardiac output, stroke volume, and mean arterial pressure compared with the dobutamine and the placebo groups.

Conclusion: Levosimendan improved hemodynamic function and survival in this animal model of severe propranolol intoxication. The potential clinical application of levosimendan in propranolol intoxication warrants further investigation. [Ann Emerg Med. 2009;54:811-817.]

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0196-0644/\$-see front matter

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doi:10.1016/j.annemergmed.2009.06.512

INTRODUCTION

Background

More than 9,000 toxic exposures to β -blockers were reported to US poison centers in 2007. Of those exposures, 692 (7.4%) were associated with severe bradycardia, hypotension, and acute negative inotropy; 3 deaths were associated with β -blocker overdose. The majority of the exposures were unintentional (86.2%), but a significant (10.8%) proportion of cases were suicide attempts.¹

The flow of calcium across myocardial cell membranes is necessary for cardiac automaticity, pulse conduction, and contraction. β -blockers indirectly decrease cyclic adenosine monophosphate production, with a subsequent decrease of calcium influx through L-type calcium channels. Interruption of calcium flux leads to decreased intracellular calcium ion concentration; this

produces cardiovascular dysfunction because of negative inotropy, which can result in cardiovascular collapse.²

Importance

The therapeutic goal in the treatment of β -blocker intoxication is to support myocardial function and thereby restore critical organ perfusion. Various treatments have been attempted, including volume expansion, atropine, cardiac pacing, insulin, dopamine, dobutamine, glucagon, enoximone, hemodialysis, continuous venovenous hemodiafiltration, and gastrointestinal decontamination with activated charcoal.³⁻⁶

The calcium sensitizer levosimendan is a targeted therapy for the treatment of acute decompensated heart failure. It is available in more than 40 countries but not yet in the United

Editor's Capsule Summary

What is already known on this topic

Severe β -blocker poisoning is treated with multiple pharmacologic agents, with limited success. Levosimendan increases the sensitivity of cardiac myocyte contractile proteins to calcium.

What question this study addressed

The hemodynamic effects of levosimendan were tested in a porcine animal model of severe β -blocker intoxication.

What this study adds to our knowledge

Compared with dobutamine and saline solution treatments, the levosimendan group showed improved survival, cardiac contractility, cardiac output, and mean arterial pressure. All 9 pigs in the levosimendan group survived, whereas 4 of 6 and 7 of 9 pigs died in the placebo and the dobutamine groups, respectively.

How this might change clinical practice

It will not yet, but levosimendan offers a promising new approach for the treatment of severe β -blocker poisoning.

States. By increasing calcium sensitivity of contractile proteins, levosimendan induces a positive inotropic effect mediated through calcium-dependent binding to troponin C.^{7,8} Levosimendan has a direct contractility-enhancing effect⁹ and also improves function of the stunned myocardium.¹⁰ Levosimendan causes coronary dilation and systemic vasodilatation through opening of adenosine triphosphatase-sensitive potassium channels.^{11,12} It seems that levosimendan works under extreme systemic conditions, such as acidosis and sepsis.^{13,14}

Goals of This Investigation

Our study assessed the effect of levosimendan on hemodynamic performance in a porcine model of propranolol-induced negative inotropy.

MATERIALS AND METHODS

Study Design

The National Animal Ethics Committee of Finland approved this methodology. Animal care, welfare, and procedures were carried out in accordance with the regulations of the Council of Europe.

Twenty-four landrace pigs (median 29 kg; interquartile range (28, 30)) were fasted, but allowed access to water, for 12 hours before the experiments. Premedication with medetomidine 50 μ g/kg, midazolam 0.10 mg/kg, ketamine 10 mg/kg, and

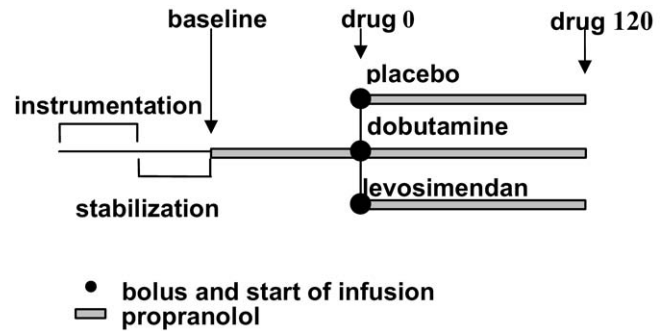


Figure 1. Experimental protocol.

fentanyl 5 μ g/kg intramuscularly was followed by cannulation of an ear vein and intravenous administration of propofol 2 mg/kg for intubation. Anesthesia was maintained with propofol (10 mg/kg per hour) and fentanyl (3 μ g/kg per hour). The animals were ventilated in volume-controlled mode (Servo 900; Siemens, Elema AB, Solna, Sweden) with 5 cm H₂O of positive end-expiratory pressure. The fraction of inspired oxygen was maintained between 0.3 and 0.6 to keep PaO₂ levels between 13.3 kPa (100 mm Hg) and 20 kPa (150 mm Hg). Tidal volume was kept at 10 mL/kg, and the minute ventilation was adjusted to maintain PaCO₂ levels between 4.5 and 5.5 kPa (34 to 41 mm Hg).

Interventions

A right femoral artery was cannulated and a pulmonary artery catheter (7.5F flow-directed, Arrow; Arrow International Inc., Reading, PA) introduced through the right internal jugular vein. The angiography catheter was inserted into the left ventricle through the left femoral artery to measure left ventricular pressures. A femoral vein was cannulated for fluid administration. During instrumentation, the animals received 3 mL/kg per hour infusions of 0.9% saline solution and 1.5 mL/kg per hour gelatin (Gelofusine; B. Braun Medical, Melsungen, Germany). Additional saline solution was administered if necessary to keep pulmonary artery occlusion pressure between 5 and 8 mm Hg. Body temperature of the animals was kept above 38°C (100°F) using an operating table heater and warmed fluids.

After instrumentation, a stabilization period of at least 30 minutes was followed by baseline measurements (Figure 1). Propranolol intoxication was then induced. The target was 40% of baseline cardiac output for 15 minutes. The intoxication model was initiated with 1 mg/kg of propranolol intravenously, followed by an intravenous infusion containing 5 mg/mL of propranolol at 180 mL/hour until target cardiac output was reached. Thereafter, the rate of propranolol infusion was reduced to 90 mL/hour and maintained until the end of the experiment. This method was chosen to mimic oral intake and was based on a pilot trial of 3 pigs.

After target cardiac output was reached, the pigs were randomized into 3 groups: 6 pigs in the placebo group, 9 pigs in

the dobutamine group, and 9 pigs in the levosimendan group. Randomization (sealed opaque envelopes) was performed by a research nurse at the Department of Intensive Care at Kuopio University Hospital. The research nurse also prepared and diluted the study drugs, thereby ensuring that the investigators and research assistants were blinded to the group assignment. All 3 groups received a 10 mL fluid bolus within 2 minutes, followed by an infusion of 0 to 15 mL/hour, which was adjusted to maintain mean arterial pressure above 65 mm Hg.

The placebo group received a 10 mL fluid bolus consisting of saline solution colored with thiamine, followed by the same saline solution infusion. The dobutamine group received a 10 mL bolus of saline solution colored with thiamine and a 2.5 mg/mL dobutamine infusion. The levosimendan group received a bolus with 1.25 mg of levosimendan in 10 mL of 5% glucose (Simdax; Orion Pharma, Espoo, Finland) and a saline solution infusion. All the fluids were similar in appearance.

Methods of Measurement

Left ventricular pressure, mean arterial pressure, central venous pressure, and pulmonary artery occlusion pressure were recorded with quartz pressure transducers and displayed continuously on a multimodular monitor (S/5 Compact Critical Care Monitor; Datex-Ohmeda, Helsinki, Finland). All pressure transducers were calibrated simultaneously and zeroed to the level of the heart. The inotropic effect was measured as a change in the maximum of the positive slope of the left ventricular pressure. It was recorded once a minute, and the mean value during 5 minutes was calculated to be used in the analysis. Cardiac output was measured by bolus injectates in triplicate with 10 mL of room-temperature 0.9% sodium chloride. The mean value of 3 measurements was used. Systemic vascular resistance was calculated from maintained mean arterial pressure, central venous pressure, and cardiac output. Pulse rate was measured from the ECG, which was also continuously monitored (Datex-Ohmeda). A median value of 2 minutes is presented in the "Results."

Blood samples were drawn at baseline, when the target cardiac output was reached (drug 0), and immediately before clinically estimated collapse of hemodynamics or at the end of the experiment (drug 120).

Primary Data Analysis

Sample size was estimated as 6 animals per group, with a 2-sided *P* level of .05 and a power of 0.80 to detect a 1 L/minute difference in cardiac output, with SD of 0.7 between levosimendan and placebo groups at 30 minutes after bolus of levosimendan or placebo. We chose a larger sample size for the treatment groups to detect possible differences between levosimendan and dobutamine groups. The difference in cardiac output and the SD were based on a previous study of calcium channel blockade intoxication in a pig model (unpublished data).

The outcome measures were normally distributed, as tested by the Kolmogorov-Smirnov test at points when there were

Table 1. Measures of hemodynamic performance.

	Baseline	Drug 0
MAP, mm Hg		
Levosimendan	110 (105; 113)	60 (49; 68)
Dobutamine	105 (105; 111)	63 (42; 66)
Placebo	107 (104; 111)	53 (49; 62)
CO, L		
Levosimendan	4.6 (4.3; 4.8)	2.3 (2.1; 2.5)
Dobutamine	4.6 (3.8; 4.6)	2.1 (1.8; 2.4)
Placebo	4.7 (4.6; 5.0)	2.3 (1.5; 2.4)
LV dP/dt, mm Hg/s		
Levosimendan	2,230 (2,008; 2,418)	621 (600; 834)
Dobutamine	2,058 (1,886; 2,140)	831 (493; 885)
Placebo	2,090 (1,868; 2,266)	693 (490; 760)
Pulse rate, beats/min		
Levosimendan	94 (86; 101)	93 (91; 96)
Dobutamine	97 (95; 103)	98 (89; 101)
Placebo	134.5 (128.5; 140.5)	100 (85.25; 108)
CVP, mm Hg		
Levosimendan	8 (7; 9)	10 (9; 10)
Dobutamine	7 (7; 9)	10 (10; 14)
Placebo	8 (8; 8)	11 (10; 11)
SV, mL		
Levosimendan	47 (42; 50)	24 (23; 28)
Dobutamine	40 (37; 46)	21 (18; 24)
Placebo	37 (34; 40)	22 (17; 23)
SVR, dyn-s/cm⁵		
Levosimendan	1,849 (1,744; 1,888)	2,131 (1,967; 2,394)
Dobutamine	1,855 (1,710; 2,091)	2,091 (1,681; 2,674)
Placebo	1,668 (1,608; 1,776)	2,090 (1,962; 2,242)

MAP, Mean arterial pressure; CO, cardiac output; LV dP/dt, maximum of the positive slope of the left ventricular pressure; CVP, central venous pressure; SV, stroke volume; SVR, systemic vascular resistance at baseline and drug 0 (median and interquartile range).

more than 3 animals alive. When there were fewer than 3 animals alive, the distribution was not tested. The mixed models were used to examine the differences between the study groups from preintoxication (baseline) to postintoxication (from drug 0 to drug 120) phases. Time point and group were treated as fixed effects (factors) and subject (pig) as a random effect. Models were constructed separately for each parameter (response variable). Estimated marginal means were calculated from the model parameters, and post hoc comparisons were based on these estimated marginal means and were Bonferroni corrected to account for multiple testing. Analyses were not adjusted for baseline differences. Values are presented as medians and interquartile ranges. The statistics and *P* values are for the living animals. The effects of propranolol intoxication are presented as percentage change from the baseline to the drug 0 level. Statistical analyses were performed with SPSS for Windows, version 16.0 (SPSS Inc., Chicago, IL). *P* values less than .05 were considered statistically significant.

RESULTS

There was no difference between the groups in baseline data, except in hemoglobin and pulse rate (Tables 1 and 2). Hemoglobin level was higher in the dobutamine group, at 74

Table 2. SvO₂, calcium, and lactate values at baseline, drug 0, and drug 120 or just before hemodynamic collapse.

	Median (Interquartile Range)		
	Baseline	Drug 0	Drug 120
SvO₂, %			
Levosimendan	69 (68; 72)	40 (32; 45)	56 (44; 63)*
Dobutamine	70 (67; 70)	39 (38; 41)	22 (19; 40)
Placebo	65 (61; 71)	30 (26; 39)	22 (19; 34)
Calcium, mmol/L			
Levosimendan	1.33 (1.29; 1.35)	1.25 (1.24; 1.34)	1.33 (1.26; 1.34)
Dobutamine	1.34 (1.26; 1.37)	1.30 (1.26; 1.30)	1.27 (1.24; 1.35)
Placebo	1.32 (1.32; 1.34)	1.29 (1.29; 1.32)	1.26 (1.17; 1.28)
Lactate, mmol/L			
Levosimendan	0.6 (0.5; 0.7)	1.20 (0.60; 1.20)	0.5 (0.5; 0.6)*
Dobutamine	0.6 (0.6; 0.8)	1.20 (1.10; 1.70)	2.0 (1.2; 2.5)
Placebo	0.6 (0.5; 0.8)	1.45 (0.8; 2.0)	1.9 (1.7; 2.1)

* $P < .05$ levosimendan versus dobutamine and levosimendan versus placebo. There were 9 animals in the levosimendan and the dobutamine groups and 6 in the placebo group.

g/L (71; 78), versus the placebo group, at 58 g/L (54; 61). Pulse rate was higher in the placebo group, at 135 beats/min (129; 141), versus the levosimendan group, at 94 beats/min (86; 101), and dobutamine group, at 97 beats/min (95; 103).

The hemodynamic parameters and laboratory data of ionized calcium, lactate, and mixed venous saturation (SvO₂) at the time point when intoxication was complete (drug 0) are presented in Tables 1 and 2. When intoxication with propranolol was complete, there were no differences between the groups except in hemoglobin between the dobutamine and placebo groups: 73 g/L (67; 76) versus 53 g/L (48; 55), respectively (Tables 1, 2). In each animal, cardiac output decreased at least by 40%: in the levosimendan group, the reduction was 50%; in the dobutamine group, 54%; and in the placebo group, 51%.

The group receiving levosimendan had higher cardiac output, maximum of the positive slope of the left ventricular pressure, stroke volume, mean arterial pressure, and pulse rate compared with the dobutamine and placebo groups at each time point (Figure 2; $P < .05$ for each time point). There was no difference in systemic vascular resistance between the groups throughout the study ($P =$ not significant). The median dose of dobutamine was 6.9 μ g/kg per minute (6.8; 19.5) during the experiment (Appendix E1, available online at <http://www.annemergmed.com>).

The final blood samples were taken immediately before the clinically estimated collapse of hemodynamics in each pig or at drug 120 minutes. In the blood samples, there was a decrease in SvO₂ in both dobutamine and placebo groups compared with the levosimendan group, in which SvO₂ increased from drug 0 to drug 120 (Table 2; $P = .003$ for levosimendan versus dobutamine; $P = .004$ for levosimendan versus placebo). The laboratory values of sodium, potassium, and glucose were within the normal range in all groups throughout the experiment (Appendix E1, available online at <http://www.annemergmed.com>).

All pigs in the levosimendan group survived the experiment. In contrast, 4 of 6 and 7 of 9 pigs died during the experiment in the placebo and the dobutamine groups, respectively (Figure 3).

LIMITATIONS

We used an animal model, which may not simulate oral propranolol intoxication in humans. This was a model of severe or near-fatal intoxication, and the hemodynamic changes in our animal model are similar to those seen with human propranolol intoxication.

Various rescue drugs and interventions are currently used to treat propranolol intoxication; in this model, we chose dobutamine as the control treatment, which may not be the method of choice by some physicians. Dobutamine is widely used in low cardiac output, and we chose to compare levosimendan to a clinically used inotropic drug. Comparing levosimendan in combination with other drugs or alone versus drugs or interventions other than dobutamine might be relevant.

DISCUSSION

The main finding in our study was that levosimendan provided a survival benefit compared with that of control treatments. All animals receiving levosimendan survived, whereas most animals died in the placebo and the dobutamine groups. Survival was associated with preserved myocardial function, as measured by maximum of the positive slope of the left ventricular pressure, cardiac output, and stroke volume. In contrast, we did not find evidence of excessive vasodilation in response to levosimendan. Therefore, the major beneficial effect of levosimendan may be due to its positive inotropic effect, mediated through calcium sensitization of contractile proteins.

Levosimendan enhances cardiac contraction by improving the use of available calcium, rather than by inundating the cell with excessive calcium.¹⁵ This effect can maintain or reverse cardiac contractile function, even under severe β -receptor blockade. Similar findings were reported in guinea pigs.¹⁶

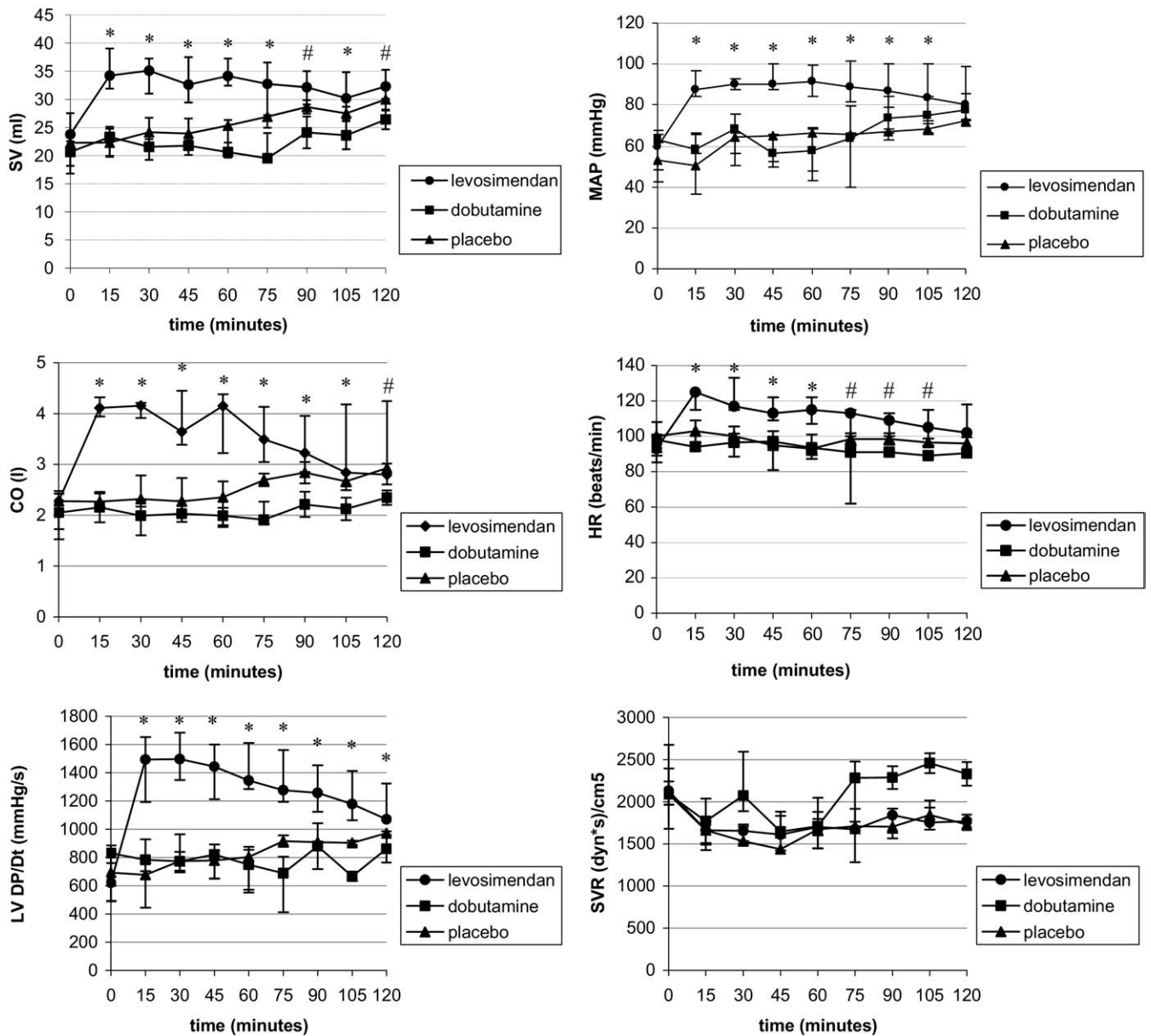


Figure 2. Hemodynamic changes in a porcine model of propranolol intoxication. Stroke volume (SV), cardiac output (CO), maximum of the positive slope of the left ventricular pressure (LV dP/dt), mean arterial pressure (MAP), pulse rate (HR), and systemic vascular resistance (SVR) between time 0 and time 120 in different groups (median and interquartile range; * $P < .05$ between levosimendan vs. placebo and dobutamine groups; # $P < .05$ between levosimendan and dobutamine groups). The statistics and P values were calculated for the animals that were alive at each time point. See “Materials and Methods” for details.

Levosimendan causes vasodilatation through opening of adenosine triphosphatase-sensitive K^+ channels,¹⁹ which may contribute to coronary and systemic vasodilation with intravenous administration of levosimendan.^{17,18} Even though the vasodilating effect of levosimendan has been well documented,¹⁹⁻²¹ it is noteworthy that in this experiment it had a positive effect on mean arterial pressure. It is conceivable that the inotropic effect of levosimendan was more prominent than the vasodilatory effect.

Dobutamine is a direct-acting sympathomimetic amine that has its primary effect on β_1 -receptors and relatively minor effects on β_2 - and α_1 -receptors. Our results indicate that nonselective blockade of β -receptors can cause dobutamine to be ineffective in reversing cardiac contractile function. This finding is supported also by previous study in humans.²² Therefore, the effect of dobutamine on inotropy in the presence of total β -blockade (intoxication) is limited. Furthermore, levosimendan was significantly more effective than dobutamine in patients on a β -blocking agent for heart failure

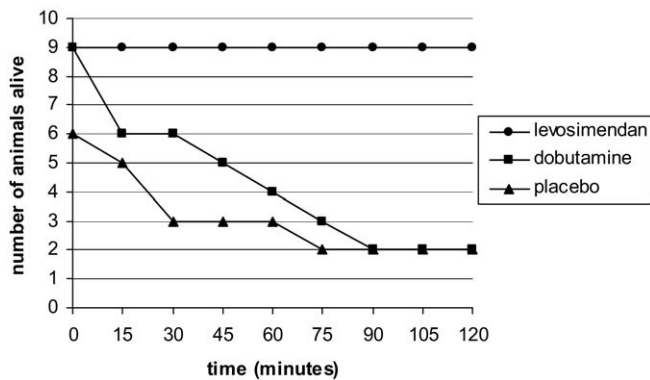


Figure 3. Animal survival between drug 0 and drug 120.

in the LIDO study.²³ Levosimendan may outcompete propranolol for β -receptor binding sites, but dobutamine cannot outcompete the propranolol.

In summary, treatment with levosimendan in this experiment improved hemodynamic measures and survival in a porcine model of propranolol intoxication. It may provide an alternative to presently recommended pharmacologic therapy in cases of severe propranolol intoxication. Confirmation of the effectiveness of levosimendan in humans requires further preclinical and clinical study. [Appendix E1](#), available online at <http://www.annemergmed.com>.

Supervising editor: Richard C. Dart, MD, PhD

Funding and support: By *Annals* policy, all authors are required to disclose any and all commercial, financial, and other relationships in any way related to the subject of this article that might create any potential conflict of interest. See the Manuscript Submission Agreement in this issue for examples of specific conflicts covered by this statement. Financial support provided by Orion Pharma Inc.

Author contributions: HL, ER, LL, and JK designed the study and obtained research funding. HL, JR, and JK performed the study. HL, VK, and JK performed statistical analysis. HL, ER, LL, and JK prepared the article. JK takes responsibility for the paper as a whole.

Publication dates: Received for publication December 23, 2008. Revisions received March 6, 2009; April 9, 2009; and April 30, 2009. Accepted for publication June 24, 2009. Available online September 19, 2009.

Reprints not available from the authors.

Address for correspondence: Kurola Jouni, MD, PhD, Department of Intensive Care, Kuopio University Hospital, PO Box 1777, 70211 Kuopio, Finland; 358-40-5677189, fax 358-17-173443; E-mail jouni.kurola@kuh.fi.

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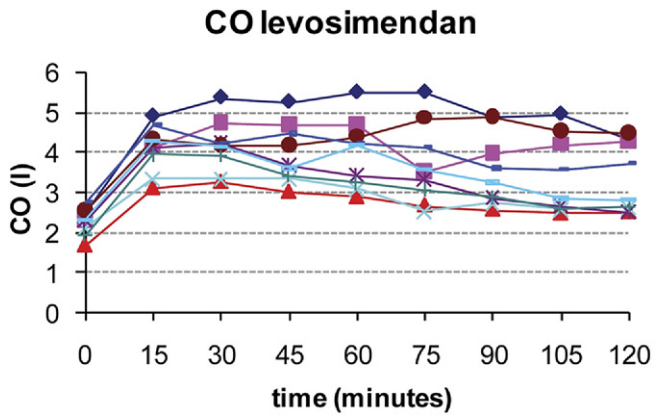


Figure E1.

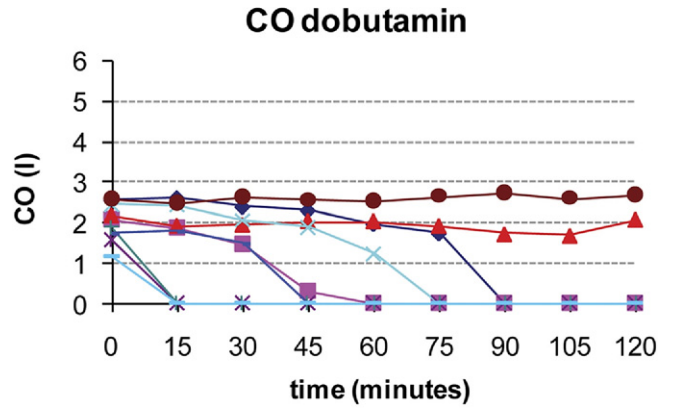


Figure E2.

APPENDIX E1. Hemodynamic changes of individual animals in a porcine model of propranolol intoxication.

The changes in CO, LVdP/dt and MAP of each animal in levosimendan, dobutamine and placebo groups from Drug 0 to Drug 120. [Figure E1-9](#)

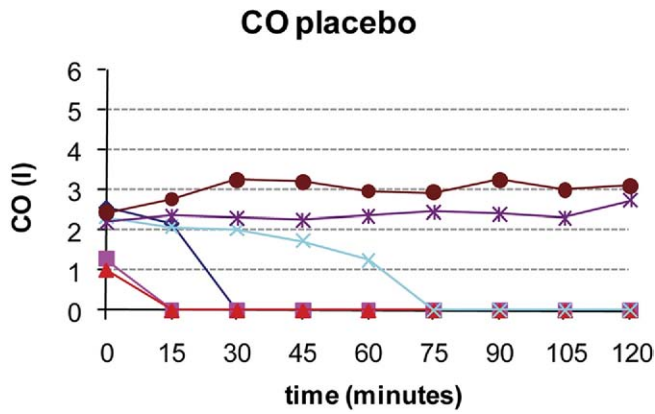


Figure E3.

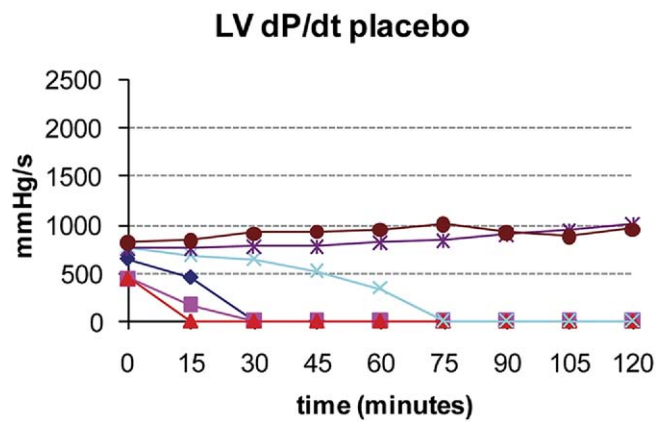


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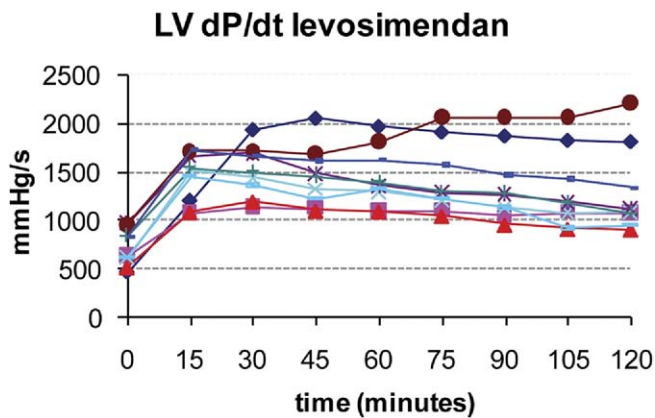


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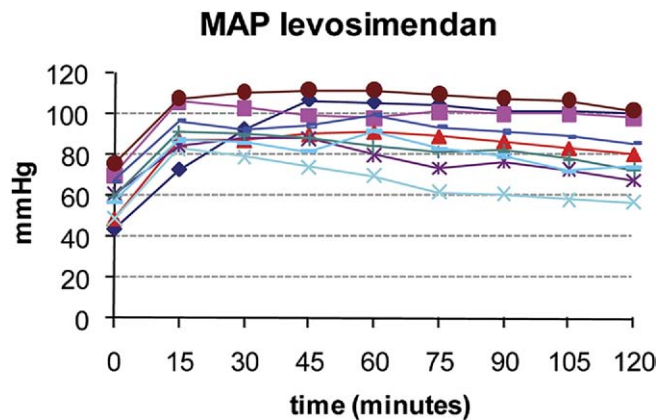


Figure E7.

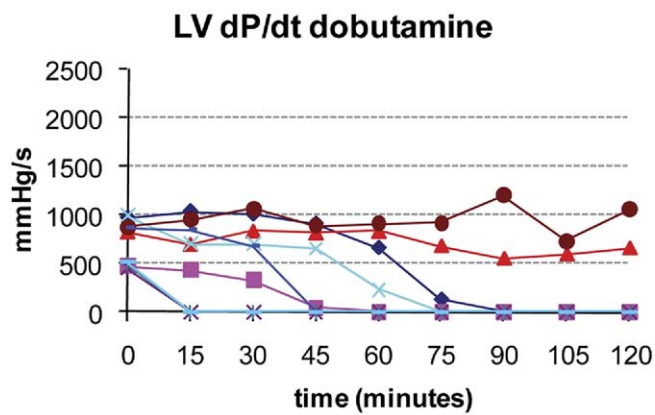


Figure E5.

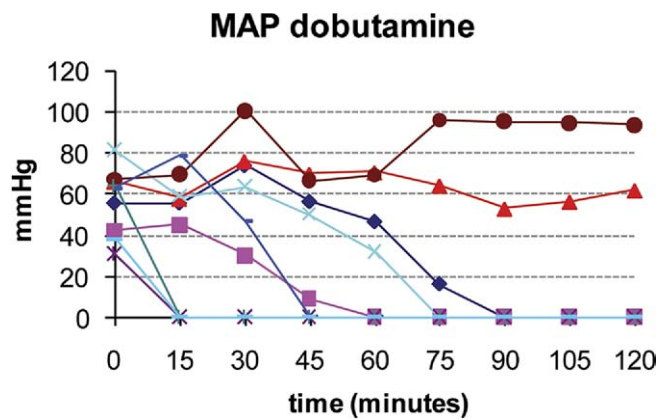


Figure E8.

MAP placebo

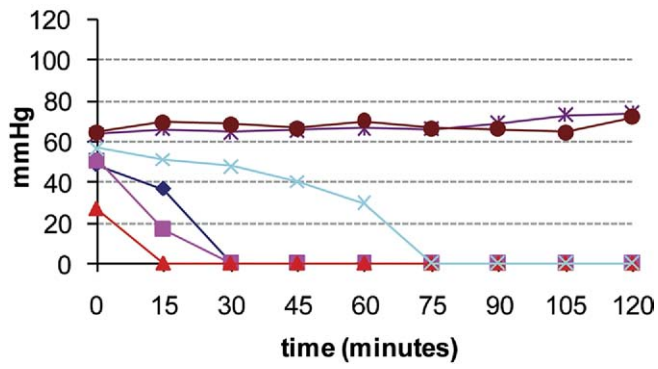


Figure E9.