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HEMODYNAMIC EFFECTS AND PHARMACOKINETICS OF LEVOSIMENDAN AND ITS METABOLITES IN PATIENTS WITH SEVERE HEART FAILURE

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ACADEMIC DISSERTATION

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Para mí familia

ABSTRACT

Levosimendan is a new drug for the treatment of decompensated heart failure. In previous studies, levosimendan has been shown to increase cardiac contractility through myofilament calcium sensitisation and induce peripheral and coronary vasodilation by opening ATP-sensitive potassium channels. The elimination half-life of levosimendan is approximately one hour in man. Preclinical studies have shown that levosimendan has an active metabolite, called OR-1896, with a substantially longer elimination half-life than that of the parent drug.

The aims of the present studies were to investigate: (1) the role of levosimendan metabolites on the hemodynamic responses achieved by levosimendan infusion and (2) the pharmacokinetics of levosimendan and its metabolites during and after intravenous levosimendan infusions of different durations in patients with severe heart failure.

Levosimendan was administered intravenously for 24 hours to 7 days in a total of 134 patients with NYHA functional class III to IV in three different study settings. Hemodynamics were followed non-invasively in all studies and also invasively in one. Blood samples for pharmacokinetic evaluations were drawn repeatedly in all studies. Safety of the patients was followed by frequent ECG recordings, safety laboratory assessments and adverse event inquiries.

Levosimendan infusion increased cardiac output and decreased pulmonary capillary wedge pressure and these effects were sustained for at least 24 hours after stopping the infusion. The effects were accompanied by an increase in heart rate and decrease in blood pressure. After the infusions were stopped, levosimendan rapidly disappeared from blood with an elimination half-life of one hour. In contrast, the plasma concentrations of levosimendan metabolites increased slowly during and even after the infusions and also decreased slowly after the infusions were stopped. The elimination half-life of the active metabolite OR-1896 was 70-80 hours. The hemodynamic effects were prolonged and the changes in hemodynamic variables closely followed the plasma concentrations of OR-1896. Levosimendan was well tolerated in the studies. However, extending the infusion duration beyond 24 hours induced a marked and long-lasting increase in heart rate.

In conclusion, the hemodynamic effects of levosimendan were prolonged beyond the infusion period. The prolongation was most likely a consequence of the formation of the active metabolite OR-1896 that has a long elimination half-life. Extending the infusion period beyond 24 hours did not bring any further benefit and was associated with prolonged increase in heart rate.

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LIST OF ORIGINAL PUBLICATIONS

I Kivikko M, Lehtonen L, Colucci WS. Sustained Hemodynamic Effects of Intravenous Levosimendan. Circulation 2003;107: 81-86.

II Kivikko M, Antila S, Eha J, Lehtonen L, Pentikäinen PJ. Pharmacokinetics of levosimendan and its metabolites during and after a 24-hour continuous infusion in patients with severe heart failure. Int J Clin Pharmacol Ther 2002;40:10 465-471.

III Kivikko M, Antila S, Eha J, Lehtonen L, Pentikäinen PJ. Pharmacodynamics and safety of a new calcium sensitizer, levosimendan, and its metabolites during an extended infusion in patients with severe heart failure. J Clin Pharmacol 2002 42:1 43-51.

IV Antila S, Kivikko M, Lehtonen L, Eha J, Heikkilä A, Pohjanjousi P, Pentikäinen PJ. Pharmacokinetics of levosimendan and its circulating metabolites in patients with heart failure after an extended continuous infusion of levosimendan. Submitted for publication.

ABBREVIATIONS

ACE	Angiotensin Converting Enzyme
AE	Adverse event
ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
ANP	Atrial Natriuretic Peptide
ATP	Adenosine Triphosphate
AUC	Area Under the Curve
BNP	Brain Natriuretic Peptide
bpm	beats per minute
cAMP	cyclic Adenosine Monophosphate
CHF	Congestive heart failure
CL_{tot}	Total Clearance
C_{max}	Maximum concentration
CO	Cardiac Output
C_{ss}	Concentration at steady state
CV	Coefficient of Variation
DBP	Diastolic Blood Pressure
FDA	Food and Drug Administration
HR	Heart Rate
LVEF	Left Ventricular Ejection Fraction
NS	Not Significant
NYHA	New York Heart Association
PAP	Pulmonary Artery Pressure
PCWP	Pulmonary Capillary Wedge Pressure
pK_a	Negative Logarithm of an Equilibrium Constant
PDE	Phosphodiesterase
PTCA	Percutaneous Transluminal Coronary Angioplasty
PVR	Pulmonary Vascular Resistance

RAP	Right Atrial Pressure
SBP	Systolic Blood Pressure
SEM	Standard Error of Mean
SD	Standard Deviation
SV	Stroke Volume
SVR	Systemic Vascular Resistance
$t_{1/2el}$	Terminal elimination half-life
T_{max}	Time to maximum concentration
V_d	Volume of distribution

1. INTRODUCTION

Congestive heart failure is a major cardiovascular disorder the incidence and prevalence of which are increasing. The financial burden of the disease to the health care system is enormous. In the United States alone, estimations of the total direct costs of care for heart failure have varied from 10 to 38 billion dollars per year (1, 2). ACE inhibitors (3) and beta-blockers (4-7) and recently also spironolactone (8) have been shown to have a beneficial effect on survival and on need for hospitalisations in patients with chronic systolic heart failure.

New orally administered inotropic agents were introduced in the 1980s and 1990s and great enthusiasm followed their appearance. However, eventually these agents were shown to increase mortality and hospitalisations in patients with congestive heart failure (9-14). Only digitalis has been shown to have some beneficial effects on morbidity and neutral effects on mortality in these patients (15).

The safety of the intravenous formulations of inotropic agents has also been under the magnifying glass in recent years. There is no direct proof that these agents increase mortality, but neither is there indisputable proof of beneficial effect on symptoms of heart failure (16, 17). Thus far the largest trial with an intravenous inotrope was conducted with milrinone, a phosphodiesterase III inhibitor, in 951 patients. The study failed to show any outcome benefit of milrinone over placebo. Moreover, the adverse events were more frequent in the milrinone group (18). Based on the disappointing results with intravenous inotropes, the treatment guidelines in Europe and USA recommend the use of these agents only as a bridging treatment for curative therapy (such as transplantation) in the most severe cases and do not recommend the use of oral inotropic agents (except digoxin) in any case (19, 20).

Levosimendan is a new option in the treatment of decompensated heart failure. Levosimendan has two main mechanisms of action: it enhances cardiac contractility through myofilament calcium sensitisation (21-25) and induces peripheral and coronary vasodilation by opening ATP-sensitive potassium channels (26-28). With higher concentrations it is also a selective phosphodiesterase inhibitor (29, 30). Cardiac performance is improved with no significant increases in oxygen consumption (31-33) or potentially malignant rhythm disorders (34). In contrast to other contractility-improving agents, levosimendan may have beneficial long-term effects on survival (35-37). This, however, has not been tested in a large-scale trial with mortality as the primary variable of interest.

Levosimendan itself has an elimination half-life of one hour (38-40). However, the beneficial effects of levosimendan treatment last longer than could be anticipated on the basis of the relatively rapid elimination of the parent drug (36). The aim of this thesis was to study the hemodynamic effects of levosimendan infusion in patients with severe congestive heart failure with special emphasis on the role of the main metabolites of levosimendan. Furthermore, the pharmacokinetics of levosimendan and the main metabolites were studied.

2. REVIEW OF THE LITERATURE

A respected textbook of cardiovascular medicine, "Heart Disease" by Braunwald, describes heart failure as follows: "Heart failure is the pathophysiological state in which the heart is unable to pump blood at a rate commensurate with the requirements of the metabolizing tissues or can do so only from an elevated filling pressure. It is usually, but not always, caused by a defect in myocardial contraction, i.e. by myocardial failure. However, in some patients with heart failure, a similar clinical syndrome is present, but there is no detectable abnormality of myocardial function. In many of such cases heart failure is caused by conditions in which the normal heart is suddenly presented with a load that exceeds its capacity or in which ventricular filling is impaired" (41).

The description includes the two main forms of heart failure, namely systolic and diastolic heart failure. In the former, coronary heart disease, for example, has damaged a remarkable proportion of heart muscle leading to a severe pump failure. In diastolic failure, the ability of the heart to receive blood is diminished for example due to stiffening of the left ventricular wall. Systolic and diastolic heart failure are not always easy to differentiate and often both components play an important role in the development of heart failure (42, 43). A rough but in clinical practice useful way to separate the two forms is the determination of left ventricular ejection fraction. Patients with signs and symptoms of heart failure and an ejection fraction below 45% probably have systolic heart failure and those with ejection fraction above 45% diastolic heart failure (44). Patients with predominantly diastolic heart failure are typically older, of female sex and have higher systolic blood pressure than patients with low ejection fraction. The latter are more often male and tend to be younger (45).

2.1 Epidemiology of heart failure

The incidence and prevalence of heart failure increase progressively with age. The Framingham study observed that the incidence of heart failure was approximately 2 per 1000 annually in those from 45 to 54 years of age and 40 per 1000 annually in those from 85 to 94 years of age with the incidence approximately doubling with each decade of age. The prevalence was about 1% in persons in their 50s and progressively increased to about 10% in persons in their 80s (46). In more recent surveys, the incidence in those over 65 years of age has varied from approximately 10 to 19 per 1000 annually (47-49). The prevalence has been shown to increase with time (50).

The most important independent risk factors for heart failure, in addition to age, are male sex, less education, physical inactivity, cigarette smoking, overweight, diabetes, hypertension, valvular heart disease, and coronary heart disease (46-51). Coronary heart disease and hypertension are the most common etiologic factors underlying the development of heart failure. It is estimated that in the USA, more than 60% of the heart failure cases in the general population might be attributable to coronary heart disease (51). The proportion of idiopathic dilative cardiomyopathy in heart failure cases in general is low; the incidence was approximately 6 per 100 000 annually in two different studies (52, 53). However, of the most severe cases of systolic heart failure, the proportion seems to be much higher. In a series of 3787 patients with a left

ventricular ejection fraction below 40% undergoing coronary angiography, nonischemic heart failure was diagnosed in 17.8% (54).

The prognosis of heart failure is poor as shown in Table 1. In the Framingham study, 37% of men and 33% of women died within 2 years of diagnosis. The 6-year mortality rate was 82% for men and 67% for women (46). In a more recent survey in the Netherlands (the Rotterdam Study), one-year mortality was 11%, 2-year 21% and 5-year 41% (55). The prognosis in those with a new diagnosis of heart failure is even poorer. A US survey in Minnesota showed a 3-month mortality of 14%, one-year mortality of 24% and 5-year mortality of 65% in those with first diagnosis of heart failure (56).

The prognosis is also dependent on the left ventricular function. In patients with preserved systolic function and thus diastolic heart failure, mortality seems to be approximately one half of the mortality in those with low left ventricular ejection fraction (57, 58). The EPICAL study evaluated patients with advanced heart failure. The criteria for inclusion into the study consisted of hospitalisation due to NYHA III-IV symptoms, radiological and/or clinical signs of pulmonary congestion and/or signs of peripheral edema, left ventricular ejection fraction <30% or a cardiothoracic ratio >60%. In these patients, the one-year mortality rate was 35% and the rate of mortality and/or readmission to hospital 81% (59).

Table 1. Prognosis of heart failure in different epidemiologic studies.

Study/Published (ref.)	**Population**	**Mortality**				
		3-month	**1-year**	**2-year**	**5-year**	**6-year**
Framingham/1991 (46)	Males with HF			37%		82%
	Females with HF			33%		67%
Minnesota/1998 (56)	First diagnosis of HF	14%	24%		65%	
EPICAL/1999 (59)	Advanced HF		35%			
Rotterdam/2001 (55)	HF		11%	21%	41%	

HF = heart failure

The in-hospital survival of heart failure patients has improved probably due to improved therapeutic tools from the 1970s to 1990s and from 1980s to 1990s but one-year survival after discharge was similar (60, 61). In a Scottish survey, however, both the short-term and long-term survival of patients with heart failure improved from the 1980s to 1990s (62).

There are several possible explanations for the increased incidence and prevalence of heart failure. The average life span has increased in the Western countries thus increasing the likelihood of developing heart failure in an individual. On the other hand, the treatment of hypertension and coronary heart disease has improved and

patients who earlier would have died of acute myocardial infarction, or stroke in the case of hypertension, now live but with a substantially damaged heart and are thus predisposed developing heart failure (63).

The long-term prognosis of patients with heart failure has also not improved as much as the appearance of new therapeutic agents would lead one to expect. A plausible explanation is that many of the patients are not treated according to current guidelines and for example ACE inhibitors and beta-blockers are not routinely used or their dose is insufficient (56, 57, 59, 63-67).

2.2 Drug therapy in chronic heart failure

ACE inhibitors

The Guidelines of the European Society of Cardiology (19) and the Guidelines of the American College of Cardiology and American Heart Failure Association (20) recommend ACE inhibitors as the first-line therapy in patients with reduced systolic function (LVEF <40-45%) even if they do not have symptomatic heart failure. The recommendation is based on several mortality studies in which ACE inhibitors have shown their efficacy.

The first study to show dramatic benefits from ACE inhibition was the Cooperative North Scandinavian Enalapril Survival Study (CONSENSUS-I), in which a 31% decrease in mortality was observed in 253 patients with severe NYHA IV heart failure at the end of 1 year of enalapril treatment when compared to placebo (p=0.001) (68). The second large trial, the SOLVD study with an average follow-up of 41 months, examined 2569 patients of whom about 90% had NYHA II-III heart failure. Enalapril was associated with a 16% risk reduction in all-cause mortality when compared to placebo (p=0.0036) (69).

Thereafter several studies have confirmed the beneficial effects of ACE inhibitors on both mortality and morbidity in heart failure. A thorough meta-analysis of the effects of ACE inhibitors in heart failure was published in 1995 (3). The analysis was based on 7105 patients from 32 trials. Six trials involving 697 patients used captopril, seven trials with 3381 patients used enalapril, six trials with 1227 patients used ramipril, five trials with 875 patients used quinapril hydrochloride, and four trials with 546 patients used lisinopril. The remaining agents, benazepril hydrochloride, cilazapril, and perindopril, were used in one or two trials with a total of 379 patients.

The primary outcome of interest in the majority of the trials (25 of 32 trials) was either symptomatic efficacy or exercise tolerance. In the two largest trials, SOLVD and CONSENSUS, the primary end-point was mortality. The remaining five trials (all using captopril) were smaller trials with mortality and/or morbidity as the outcome of interest.

Since the majority of the trials examined the effect of treatment on exercise capacity, they were usually of short duration (follow-up of 3 to 6 months). The SOLVD trial had the longest average duration of follow-up of 41 months. The patients had systolic heart failure with ejection fraction below 35-40% in the majority of the trials and NYHA functional class II-IV (in most patients NYHA II-III).

Overall, there were 611 deaths among 3870 (15.8%) patients allocated to the ACE inhibitor group and 709 deaths among 3235 controls (21.9%), indicating a statistically significant reduction in mortality (Odds ratio 0.77; 95% confidence interval 0.67-0.88). Also the combined endpoint of mortality or hospitalization for congestive heart failure was reduced by the treatment with ACE inhibitors (Odds ratio 0.65; 95% confidence interval 0.57-0.74). Similar benefits were observed with several different ACE inhibitors, although the data were largely based on enalapril, captopril, ramipril, quinapril hydrochloride, and lisinopril.

Reductions in total mortality and the combined endpoint were similar in various subgroups including age, sex, etiology, and NYHA class. However, patients with the lowest ejection fraction appeared to have the greatest benefit. The greatest effect was seen during the first 3 months, but additional benefit was observed during further treatment. The reduction in mortality was primarily due to fewer deaths from progressive heart failure (Odds ratio 0.69; 95% confidence interval 0.58-0.83); point estimates for effects on sudden or presumed arrhythmic deaths were less than 1 but not significant.

Diuretics

There is no data on hard end-points including mortality and hospitalisations with the use of diuretics (loop or thiazide type) in heart failure. Both the European and US guidelines recommend the use of loop diuretics, thiazides and metolazone according to clinical need in cases with fluid retention. The guidelines do not recommend routine use of spironolactone or other potassium-sparing agents due to the risk of hyperkalemia in concomitant use with ACE inhibitors in NYHA I-II patients. However, due to a recent study with small dose spironolactone (the RALES study) showing improved survival when compared to placebo in patients on ACE inhibitors and diuretics (8), the guidelines recommend the use of spironolactone in NYHA III-IV patients.

Beta-blockers

Guidelines recommend the use of beta-blockers in patients with ischemic or non-ischemic cardiomyopathy with reduced systolic function in NYHA II-IV patients and also in asymptomatic patients with reduced systolic function after acute myocardial infarction.

As for ACE inhibitors, the benefits of beta-blockers on survival have been clearly shown in several large-scale mortality studies. In a study in 1094 patients with mostly mild to moderate symptoms of heart failure, carvedilol decreased the risk of death by 65% (4) and in 2289 patients with severe symptoms by 35% (5) when compared to placebo. The CIBIS-II study demonstrated the beneficial effect of bisoprolol on survival in 2647 patients with NYHA III-IV heart failure; the hazard ratio for death was 0.66 (95% confidence interval 0.54-0.81, $p<0.0001$) in bisoprolol treated patients when compared to placebo (6). Metoprolol decreased all-cause mortality by 34% in comparison to placebo in NYHA II-IV heart failure patients (7).

No superiority over placebo was, however, seen with bucindolol in a study in 2708 patients of whom the majority were in NYHA class III. During the average follow-up of 2 years, mortality was 33% in the placebo group and 30% in the bucindolol group (p=0.13) (70). There is no clear explanation why bucindolol, unlike the other beta-blockers, failed to show mortality benefit over placebo. The study population was similar to the populations in other beta-blocker trials and therefore the result may be related to the compound itself.

Angiotensin II receptor antagonists

Angiotensin II receptor antagonists are recommended for those who do not tolerate ACE inhibitors. The beneficial effects of these agents on survival have not been shown indisputably.

The ELITE I study in elderly patients suggested that the angiotensin II antagonist losartan might be associated with improved survival when compared with captopril, an ACE inhibitor (71). To confirm the promising results, the ELITE II study recruited 3152 patients aged 60 years or older with NYHA II-IV heart failure and ejection fraction of 40% or less. However, during the median follow-up of 555 days, the mortality rate was slightly, although not significantly, higher in the losartan group; 11.7 versus 10.4% average annual mortality rate (hazard ratio 1.13; 95.7% confidence interval 0.95-1.35, p=0.16). Losartan was, however, better tolerated as significantly fewer patients in the losartan group (excluding those who died) discontinued study treatment because of adverse effects (9.7 vs 14.7%, p<0.001), including cough (0.3 vs 2.7%) (72).

In a recently published large trial 5010 NYHA II-IV heart failure patients were randomly assigned to receive valsartan or placebo (the Val-HeFT study). There was no difference in mortality although in the combined end-point of mortality and morbidity (defined as the incidence of cardiac arrest with resuscitation, hospitalization for heart failure, or receipt of intravenous inotropic or vasodilator therapy for at least four hours), the incidence was 13.2% lower in the valsartan group (relative risk 0.87; 97.5% confidence interval 0.77-0.97, p=0.009). In a *post hoc* analysis of the combined end point and mortality in subgroups defined according to baseline treatment with ACE inhibitors or beta-blockers, valsartan had a favorable effect in patients receiving neither or one of the two types of drug but an unfavorable effect in patients receiving both types of drugs (73).

Cardiac glycosides

Cardiac glycosides (digitalis or digitoxin) are recommended in heart failure patients with atrial fibrillation whether systolic function of the heart is reduced or not and in patients with sinus rhythm and persistent symptoms of heart failure despite the use of ACE inhibitors and diuretics. This information is largely based on the results of the DIG study (15). Digoxin was shown to have a neutral effect on mortality in patients with chronic heart failure treated with diuretics and ACE inhibitors. 6800 of the patients had a left ventricular ejection fraction of 45% or less and 988 more than 45%. Half of the patients received digoxin (median dose 0.25 mg) and half placebo and the mean follow-up was 37 months.

In patients with the lower ejection fraction, there were 1181 deaths (34.8%) with digoxin and 1194 deaths (35.1%) with placebo (risk ratio 0.99; 95% confidence interval 0.91-1.07, p=0.80). In the digoxin group, there was a trend towards a decrease in the risk of death due to worsening heart failure (risk ratio 0.88; 95% confidence interval 0.77-1.01, p=0.06). Additionally, there were 6% fewer hospitalizations overall in that group than in the placebo group, and fewer patients were hospitalized for worsening heart failure (26.8% versus 34.7%; risk ratio 0.72; 95% confidence interval 0.66-0.79, p<0.001). In patients with an ejection fraction over 45%, the mortality rates were 23.4% in both groups (risk ratio 0.99; 95% confidence interval 0.76-1.28). There is no proven benefit from digitalis in diastolic heart failure and it should only be used in patients who also have atrial fibrillation (74).

Other oral inotropic agents

All the other oral inotropic agents – except digitalis – have shown increased mortality when compared to placebo (Table 2). The guidelines, therefore, recommend that they should not be used in heart failure patients. The outcome studies with these agents are summarised below.

Milrinone, a phosphodiesterase III inhibitor, was studied in 1088 patients with NYHA III-IV chronic heart failure (the PROMISE study). When compared with placebo, milrinone therapy was associated with a 28% increase in mortality from all causes (p=0.038). Moreover, the adverse effects of milrinone were most frequent in patients with NYHA IV heart failure (9).

Vesnarinone is both a phosphodiesterase III inhibitor and a sodium channel opener (75). Two doses of the compound were compared to placebo in a large study in 3833 patients with NYHA III-IV heart failure and a left ventricular ejection fraction of 30% or less despite optimal treatment (the VEST study) (10). The mean follow-up was 286 days. There were 242 deaths in the placebo group (18.9%), 268 in the lower dose (30-mg daily) vesnarinone group (21.0%), and 292 in the higher dose (60-mg daily) vesnarinone group (22.9%). The difference between the higher vesnarinone group and placebo was significant (p=0.002). The increase in mortality with vesnarinone was attributed to an increase in sudden death, presumed to be due to arrhythmia. The quality of life improved significantly more in the 60-mg vesnarinone group than in the placebo group at 8 weeks (p<0.001) and 16 weeks (p=0.003) after randomization.

A smaller study with enoximone, another phosphodiesterase III inhibitor, in 151 NYHA III-IV patients showed a statistically significant increase in mortality with enoximone when compared to placebo; 27 of the 75 enoximone-treated patients died whereas only 18 of the 76 placebo-treated patients died (p<0.05). However, quality of life measures were significantly better in the enoximone group (11).

Pimobendan, a phosphodiesterase inhibitor with calcium sensitizing properties, was shown to improve exercise capacity in 317 patients with chronic heart failure when compared to placebo. The two doses used in the trial, however, seemed to have a detrimental effect on survival although the difference did not reach statistical significance; both pimobendan groups combined, the mortality was 1.8 times higher than in the placebo group (95% confidence interval 0.9-3.5, p>0.05) (12).

Xamoterol, a beta-adrenergic partial agonist, increased mortality in a study in 516 patients with NYHA III-IV heart failure when compared to placebo; 32 (9.1%) patients in the xamoterol group and 6 (3.7%) patients in the placebo group died within 100 days of randomisation (p=0.02) (13).

A large mortality study with ibopamine, an orally active dopamine agonist, in NYHA III-IV heart failure, was stopped early because of excess mortality (the PRIME II study). After 1906 patients had entered the trial, 232 (24%) of 953 patients in the ibopamine group died, compared with 193 (20%) of 953 patients in the placebo group (relative risk 1.26; 95% confidence interval 1.04-1.53, p=0.017) (14).

Table 2. Mortality in placebo-controlled studies with oral inotropic agents.

Study / published (ref.)	Compound	N	NYHA class	Follow-up*	Deaths (%) active/placebo	p-value
XAMOTEROL/1990 (13)	xamoterol	516	III-IV	3 months	9 / 4	0.02
PROMISE/1991 (9)	milrinone	1088	III-IV	6 months	30 / 24	0.038
1994 (11)	enoximone	151	III-IV	up to 20 months	36 / 24	p<0.05
PICO/1996 (12)	pimobendan	317	II-III	6 months	11 / 6	NS
DIG/1997** (15)	digoxin	6800	I-IV	37 months	35 / 35	0.80
PRIME II/1997 (14)	ibopamine	1906	III-IV	12 months	24 / 20	0.017
VEST/1998 (10)	vesnarinone	3833	III-IV	10 months	21-23*** / 19	0.002

*) approximate duration
**) main trial (those with ejection fraction ≤ 45%)
***) with lower and higher dose
NS = not significant

Experimental drugs

New drugs, under evaluation for the treatment of chronic heart failure studied in large-scale mortality trials include omapatrilat and bosentan. Omapatrilat has a dual mechanism of action. It inhibits both neutral endopeptidase and angiotensin-converting enzymes. In a preliminary comparative study in 573 patients with NYHA II-IV heart failure and left-ventricular ejection fraction of 40% or less, 289 patients received omapatrilat and 284 lisinopril for 24 weeks. There was a suggestive trend in favour of omapatrilat on the combined endpoint of death or admission to hospital for worsening heart failure (hazard ratio 0.53; 95% confidence interval 0.27-1.02, p=0.052) and a significant benefit of omapatrilat in the composite of death, admission, or discontinuation of study treatment for worsening heart failure (hazard ratio 0.52; 95% confidence interval 0.28-0.96, p=0.035) (76).

The promising results led to a large multicenter study in 5770 patients with NYHA II-IV heart failure, in which omapatrilat was compared to enalapril, the OVERTURE study. The mean follow-up was 14.5 months. There was no difference in the primary end-point of “all-cause mortality or hospitalisation for heart failure requiring intravenous treatment” between the drugs; 973/2884 meeting the end-point in enalapril group and 914/2886 in the omapatrilat group (hazard ratio 0.94, 95%

confidence interval 0.86-1.03, p=0.187). Hypotension was more frequent but renal impairment less frequent with omapatrilat. Thus, the results of the study indicate that omapatrilat and enalapril have similar outcome effects in patients with chronic heart failure (77).

Bosentan is the most extensively studied endothelin receptor antagonist. It is a mixed ET(A)/ET(B)-receptor antagonist. Bosentan acutely and during short-term oral therapy markedly improved hemodynamics in patients who received standard heart failure therapy, including an ACE inhibitor (78). The REACH-1 trial demonstrated that initiation of bosentan therapy was associated with an increased risk of worsening heart failure when compared to placebo. However, at 6 months the results seemed to be in favour of bosentan (78). These controversial results led to two larger studies, ENABLE-1 in Europe and ENABLE-2 in the USA and Canada. The final results of these studies have not been published yet but have been presented in cardiological congresses. There was no benefit from bosentan over placebo. Moreover, bosentan treatment led to fluid retention significantly more often than placebo (79).

Three placebo-controlled mortality trials with agents antagonising the effects of tumor necrosis alpha were recently discontinued early. The RENEISSANCE and RECOVER studies with etanercept were discontinued for futility and a smaller study with infliximab because of high mortality and hospitalisation rates in the active therapy group (80).

A study with a prostacyclin analogue, epoprostenol, in severe systolic heart failure was terminated early because of a strong trend toward decreased survival in the patients treated with epoprostenol (81). Also a study with moxonidine in patients with heart failure was discontinued prematurely due to increased mortality in the active treatment group (82).

Summary of the pharmacological treatment options in chronic heart failure

Table 3 summarises the currently recommended pharmacological treatment of chronic heart failure. The table is based on European and US guidelines (19, 20).

Table 3. Drug therapy in chronic heart failure.

NYHA class	Basic therapy	Specific cases
I	ACE inhibitor	beta-blocker if post-AMI, digitalis if atrial fibrillation
II	ACE inhibitor + beta-blocker + diuretics (if fluid retention)	digitalis if atrial fibrillation, AT_1 blocker if ACE inhibitor intolerance
III	all above + digitalis + aldosterone antagonist	AT_1 blocker if ACE inhibitor intolerance
IV	same as with NYHA class III	AT_1 blocker if ACE inhibitor intolerance

Post-AMI = patients with previous myocardial infarction

2.3 Drug therapy in acute heart failure

Acutely decompensated heart failure usually warrants hospitalisation of a patient. Hospitalisations generate the majority of the health care costs related to heart failure (2, 83, 84). The efficacy and outcome of the medical treatment options in acutely decompensated heart failure are, however, less well documented than those in chronic heart failure. Studies have often been small and uncontrolled, concentrating on acute hemodynamic or symptomatic improvement with no focus on long-term outcome measures.

Acutely decompensated heart failure may develop in a previously asymptomatic patient for example due to massive acute myocardial infarction leading to severe pumping failure, aortic dissecation leading to massive aortic valve insufficiency, endocarditis or acute myocarditis (85). More often, however, acute decompensation occurs in a patient with previously existing heart failure. Apparently minor changes in a patient´s condition may lead to acute decompensation requiring hospitalisation. For example excessive sodium chloride intake, a seemingly minor inflammatory disease such as the common cold, or noncompliance to drug therapy are frequent reasons for decompensation in chronic heart failure patients. Myocardial ischaemia and arrhythmic events are other common underlying factors for worsening condition in these patients. In a survey in 768 patients with systolic heart failure (LVEF < 40%), factors implicating worsening symptoms of heart failure were: noncompliance with salt restriction in 22%, other noncardiac causes in 20% (e.g. pulmonary infectious processes), use of antiarrhythmic agents in the past 48 hours in 15%, arrhythmias in 13%, use of calcium channel blockers in 13% and inappropriate reductions in heart failure therapy in 10% (e.g. diuretic dose) (86).

Acutely decompensated patients present with symptoms and signs of elevated preload; rashes in pulmonary auscultation, prominent jugular veins - and especially when related to chronic heart failure - enlarged liver and peripheral oedema. Compensatory mechanisms due to sympathetic overdrive may lead to vasoconstriction as evidenced by cool or cyanotic extremities and deteriorated renal function. Chest X-ray shows either enlarged or normal sized heart with congestion or oedema. Invasive measurements show elevated pulmonary capillary wedge pressure (PCWP) and often reduced cardiac output (74).

The treatment of acute decompensation depends on the underlying cause. For example, acute massive myocardial infarction warrants revascularisation therapy – thrombolytic treatment, PTCA or cardiac bypass; acute valvular catastrophe may warrant emergency surgical treatment etc. (85). In case of worsening chronic heart failure, the pre-existing maintenance therapy is continued, although hypotension may require temporary discontinuation or dose reduction of beta-blocking agents and of ACE inhibitor (74).

Intravenous diuretics and traditional vasodilators

Rapid relief of symptoms can usually be obtained with intravenously or intramuscularly administered morphine followed by intravenous loop diuretics in boluses or infusions, sometimes supplemented with other diuretics such as metolazone. Initial improvement in congestive symptoms can be accelerated by

intravenous vasodilators such as nitroglycerin or sodium nitroprusside (19, 20, 87). No randomized prospective large-scale trials have been carried out to compare the efficacy and tolerability of various types of diuretics (88) or efficacy of intravenous diuretics to vasodilator therapy. However, in one randomized study in 110 patients, therapy with high-dose intravenous isosorbide dinitrate and low-dose intravenous furosemide was compared to low-dose intravenous isosorbide dinitrate and high-dose intravenous furosemide. It was found out that the former approach with more emphasis on the nitrate therapy was more effective, as evidenced by lower need for mechanical ventilation and lower incidence of myocardial infarction (89). In a smaller study in 32 patients, the combination of morphine and intravenous furosemide was shown as effective as intravenous nitrate infusion in acute pulmonary oedema (90).

It is not known whether diuretics or vasodilator infusions have benefits beyond early symptom relief (74, 88). However, combined diuretic and vasodilator therapy, in conjunction with normalising the loading conditions in acutely decompensated heart failure, also reduced neurohormonal activation in the short term as evidenced by markedly reduced plasma concentrations of endothelin-1, catecholamines, renin, aldosterone, angiotensin and atrial natriuretic peptides (91). The most commonly used intravenous vasodilators are nitroglycerin and sodium nitroprusside. Prolonged infusions of the former may lead to development of tolerance (92-95) and of the latter to thiocyanate toxicity (96, 97).

New vasodilators

A new vasodilating agent, recombinant human brain natriuretic peptide, nesiritide, has recently been approved by the Food and Drug Administration (FDA) for the treatment of acutely decompensated heart failure.

The VMAC study, in 489 patients with dyspnoea at rest due to decompensated congestive heart failure, evaluated the acute effects of nesiritide compared to placebo or intravenous nitroglycerin (98). At 3 hours PCWP decreased significantly more with nesiritide than with placebo (by 5.8 mmHg versus 2.0 mmHg) but similarly to nitroglycerin (by 3.8 mmHg). The symptom of dyspnoea improved significantly when compared to placebo but there was no difference to nitroglycerin. The symptom benefit was driven by the results in patients who were invasively monitored. Among patients without invasive monitoring (half of the total population), no difference in symptom relief was seen. After 3 hours, the placebo group was switched to continue with either nesiritide or nitroglycerin and at 24 hours PCWP was slightly but significantly more decreased in patients treated with nesiritide than in those treated with nitroglycerin. The 6-month mortality in the nesiritide group was 25.1% and in the nitroglycerin group 20.8% but the difference was not statistically significant ($p=0.32$).

In concordance with the vasodilating effects of nesiritide, the most common adverse drug reaction with the compound is dose-related hypotension (98-100). In the VMAC trial this occurred as often as with nitroglycerin but the duration of hypotension was significantly longer with nesiritide, probably due to its longer elimination half-life, 18 minutes versus 2.5 minutes for nitroglycerin (98).

Another new vasodilator, tezosentan, is being studied for decompensated heart failure. Tezosentan is an intravenous endothelin receptor A/B antagonist. It has an elimination

half-life of 3.2 hours (101). Infusions up to 72 hours have shown beneficial hemodynamic effects in healthy volunteers and in patients with advanced heart failure. PCWP and systemic vascular resistance have decreased significantly with tezosentan when compared to placebo. Also an increase in cardiac index has been reported but this is probably compensatory due to vasodilatory effects. The most common adverse drug reaction has been headache (102-106). The published studies have been small. Larger double-blind, placebo-controlled studies (the RITZ studies) have not been able to show symptomatic benefit with tezosentan over placebo (unpublished data).

Other agents

Intravenous inotropic therapy is used to correct hemodynamic disturbances of severe episodes of worsening heart failure. Due to the observed problems associated with traditional intravenous inotropic agents (dobutamine, milrinone, amrinone or enoximone) their use should be restricted to cases refractory to other treatment or as a bridge therapy to further treatment that will benefit the patient (19, 20, 74, 87). The new European guidelines, however, mention that levosimendan "appears to be safer than dobutamine" (74). The intravenous inotropic agents and levosimendan are described in more detail in the following chapters.

Intravenous inotropic agents

Dobutamine

Dobutamine, a sympathomimetic amine, is the most commonly used intravenous inotropic agent in advanced heart failure not responding adequately to intravenous or oral vasodilators, digitalis and diuretics. It is available for clinical use as racemic mixture that stimulates both $beta_1$ and $beta_2$ adrenergic receptor subtypes and either binds but does not activate alpha-adrenergic receptors ([+] enantiomer) or stimulates $alpha_1$ and $alpha_2$ receptor subtypes ([-] enantiomer). Lower doses resulting in a clear positive inotropic effect in humans exert a predominant $beta_1$ adrenergic effect, while alpha-adrenergic agonist effects of the (-) enantiomer in the vasculature and myocardium appear to be blocked by the alpha-receptor antagonist effect of the (+) enantiomer. In addition to having a positive inotropic effect, racemic dobutamine also acts as a vasodilator to reduce aortic impedance and systemic vascular resistance thus reducing afterload (107, 108).

The adrenergic effects of dobutamine, however, lead to increased levels of intracellular cyclic AMP and calcium (109) and this further predisposes patients to arrhythmia and ischaemia (110). Also, the down-regulation of beta-adrenergic receptors with prolonged dobutamine infusion (111) has clinical implications leading to development of tolerance (112). The elimination half-life of dobutamine is only a few minutes (113-115). This allows rapid modifications in the treatment for the desired hemodynamic responses but also means that the effects of dobutamine rapidly disappear when the infusion is stopped.

Several small uncontrolled studies have shown hemodynamic and symptomatic improvement following dobutamine at doses of 2.5 - 15 μg/kg/min given for 24 to 72

hours (116-118). Data on the use of intermittent long-term infusions have suggested that dobutamine may have an adverse effect on mortality (119, 120). In a retrospective analysis of the data from the FIRST study, dobutamine-treated patients had increased mortality in comparison with patients not treated with dobutamine (121). However, the FIRST study was not a prospective, randomised, controlled trial examining the effects of dobutamine treatment but rather of epoprostenol compared with conventional treatment in patients with advanced heart failure. It was observed that those on dobutamine at baseline had a significantly higher 6-month mortality rate than those who were not. However, it is apparent that patients who were in need of inotropic support at baseline represent patients with more severe heart failure. This is highlighted by the fact that 89% of patients treated with dobutamine were in NYHA class IV compared with 53% of patients not treated with dobutamine. Thus, the results of this *post hoc* analysis must be interpreted with caution.

Milrinone

Milrinone is the most widely used phosphodiesterase III (PDE III) inhibitor. By inhibiting the breakdown of cAMP, milrinone enhances contractility in myocytes and relaxation in smooth muscle cells (108, 122). In the setting of low-output congestive heart failure, milrinone reduces systemic and pulmonary vascular resistance, decreases diastolic filling pressure and augments stroke volume and cardiac output. Milrinone also increases ventricular compliance during diastole (lusitropic effect). Similarly to other compounds that increase cAMP concentration, milrinone increases intracellular calcium thus predisposing patients to increased risk of arrhythmia (108). The plasma concentrations of milrinone increase dose-dependently and its elimination half-life is approximately 2 hours (123). Due to the relatively long elimination half-life, a loading dose is recommended in order to get immediate hemodynamic response (124). Elimination is prolonged in renal impairment and thus caution should be exercised when milrinone is used in patients with renal insufficiency (125, 126).

The beneficial hemodynamic effects of milrinone on cardiac output, end diastolic ventricular pressures and systemic vascular resistance have been shown in several studies in patients with congestive heart failure (127, 128). When compared to dobutamine, milrinone produces similar improvement in cardiac index and exerts stronger vasodilative effects. Unlike dobutamine, milrinone does not seem to increase myocardial oxygen consumption, which is possibly explained by its substantial vasodilating capacity (129). Milrinone has also been dosed intermittently (130) and in outpatient setting with continuous infusions up to several months (124). These treatments have, however, been open-label infusions in severely ill patients and no relevant data on mortality or hospitalisations has been gathered.

A large multicenter study to evaluate the effects of intravenous milrinone in 951 patients admitted to hospital with an exacerbation of systolic heart failure not requiring intravenous inotropic support was performed in 78 US centers (18). The study was a prospective, randomized, double-blind, placebo-controlled trial in which 477 patients received a 48-hour infusion of milrinone and 472 a corresponding placebo infusion. The patients had NYHA III-IV symptoms and a mean left ventricular ejection fraction of 23%. The median number of days hospitalised for cardiovascular causes within 60 days (the primary efficacy variable) was 6 days in the milrinone group and 7 days in the placebo group (p=0.71). The milrinone-treated

patients had, however, more often sustained hypotension (10.7% vs 3.2%; p<0.001) and new atrial arrhythmias (4.6% vs 1.5%; p=0.004) The milrinone and placebo groups did not differ significantly in either in-hospital mortality (3.8% vs 2.3%; p=0.19), 60-day mortality (10.3% vs 8.9%; p=0.41), or the composite incidence of death or readmission to hospital (35.0% vs 35.3%; p=0.92).

The study thus failed to show any outcome benefit of intravenous milrinone over placebo. On the contrary, adverse events were more frequent with milrinone. Oral milrinone had earlier shown increased mortality and morbidity over placebo (9). The results of these studies have raised questions whether the use of milrinone should be withheld even in the management of a hemodynamic crisis requiring inotropic therapy (131).

Other inotropic agents

There are several other intravenous inotropic agents in addition to dobutamine and milrinone. Beta-receptor stimulants include dopamine and dopexamine. Their mechanism of action and consequently clinical effects slightly differ from that of dobutamine. As with dobutamine, the clinical trials performed with these agents are mostly small and have not been powered to measure effects on hard end-points, such as mortality or hospitalisations (132-135).

Amrinone was the first widely used PDE III inhibitor. The use of amrinone is, however, associated with development of thrombocythemia (136) and therefore milrinone has largely overtaken amrinone´s place in clinical use. Overall, the hemodynamic effects and pharmacokinetics of amrinone resemble those of milrinone (123, 137). Enoximone, another PDE III inhibitor has similar hemodynamic effects to those of milrinone and amrinone. Unlike the other PDE III inhibitors, enoximone has been shown to have an active metabolite, enoximone sulfoxide, and the pharmacokinetics are reported to be non-linear (138-140). As with beta-receptor agonists, the studies with intravenous amrinone or enoximone have been too small to give relevant information on their effects on prognosis.

Several other intravenous inotropes have been studied only in experimental animals or in small or uncontrolled clinical studies. No major new drug for clinical use has emerged from this group of compounds (30).

2.4 Levosimendan

Chemistry

Levosimendan *[(R)-[[4-(1,4,5,6-tetrahydro-4-methyl-6-oxo-3-pyridazinyl)phenyl] hydrazono]propanedinitrile]* belongs to a new class of drugs, the calcium sensitisers. The structural formula of levosimendan is presented in Figure 1. Levosimendan is a moderately lipophilic drug with a molecular weight of 280.3 daltons. It is a weak acid with pK_a 6.3. Solubility of levosimendan in distilled water and phosphate buffer (pH 8) is poor (0.04 mg/ml and 0.9 mg/ml, respectively). Solubility in ethanol is 7.8 mg/ml and therefore levosimendan in its pharmaceutical composition (Simdax®2.5 mg/ml infusion concentrate) is diluted in ethanol (141).

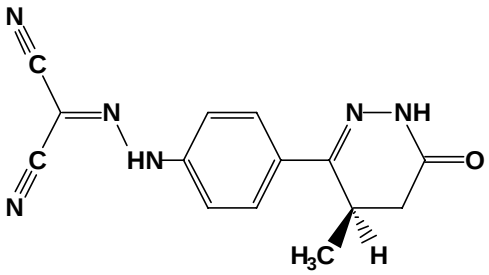

Figure 1. Structural formula of levosimendan.

Mechanism of action

Levosimendan exerts its effects through two different mechanisms: by calcium sensitisation of the contractile proteins in cardiac muscle and by opening the ATP-sensitive potassium channels both in vascular and cardiac tissue.

The positive inotropic action of levosimendan is brought about by the calcium-dependent binding of the drug to cardiac troponin C (Figure 2) (21, 23, 24, 142). Since levosimendan dissociates from cardiac troponin C at low calcium concentration (21), the compound does not impair relaxation (143, 144). Furthermore, levosimendan does not affect intracellular calcium concentrations to any great extent. It has been shown to increase contractility considerably with only a modest increase in intracellular calcium, even in ventricular muscle strips from end-stage failing human hearts (145).

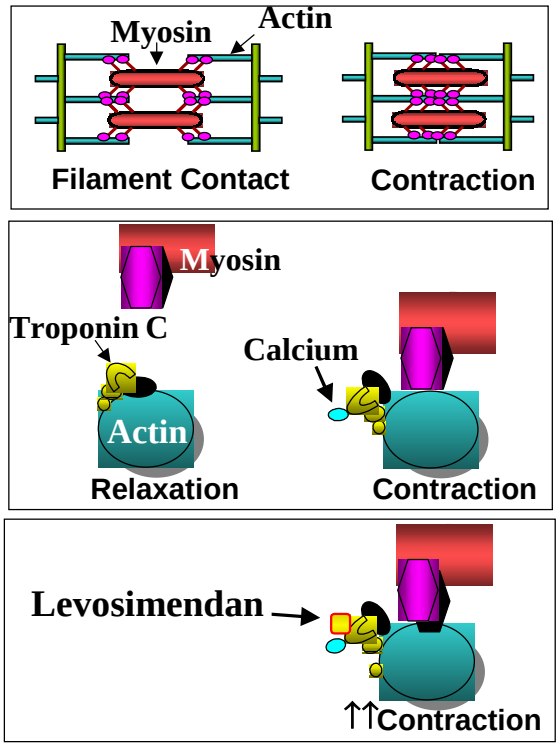

Figure 2. Calcium sensitisation of the contractile proteins in cardiac muscle by levosimendan.

The vasodilative mechanism of action is due to the opening of ATP-dependent K-channels in vascular smooth muscle (26-28). One *in vitro* study suggested that accumulation of cAMP may possibly participate in vasorelaxation at higher concentrations with levosimendan, but a cAMP-independent mechanism seems to be involved at lower concentrations (146).

In vitro studies indicate that doses of levosimendan considerably greater than those recommended in clinical practice cause highly selective inhibition of PDE III compared with the other PDE isoenzymes (30). However, this effect does not contribute significantly to the contractility-enhancing and vasodilatory effects of levosimendan in isolated guinea-pig heart (29) or to force production in man even under stimulation of cAMP synthesis by isoprenaline (147).

Levosimendan has an active metabolite, OR-1896. Similarly to levosimendan, OR-1896 exerts its positive inotropic effect on myocardium through a direct calcium sensitising effect on the contractile proteins (148, 149). The metabolite has also vasodilating effects, but the underlying mechanisms are not yet known.

Clinical effects in healthy volunteers

For ethical reasons, the hemodynamic effects of levosimendan in healthy volunteers have only been studied using non-invasive methods. Most of the studies were conducted using short infusions of 5-10 minutes. Generally no increases in heart rate have been seen with doses of up to 1 mg of levosimendan (40, 150, 151). Levosimendan has been shown to increase cardiac output and ejection fraction dose-dependently (150). The increase in cardiac output at low doses was due exclusively to

an increase in stroke volume. In most of the studies, heart rate increased at levosimendan doses of 2 mg and above, thus contributing to the increased cardiac output.

The enhancement in cardiac performance was attributed both to an increase in contractility (assessed with systolic time intervals (40, 151, 152) and echocardiography (150)) and to a reduction in afterload, reflected by a dose-dependent decrease in systemic vascular resistance (40, 150, 151). The effects of levosimendan on cardiac performance were maintained during light dynamic exercise (151). Systolic blood pressure generally increased or was unchanged, and diastolic blood pressure has consistently been shown to decrease (40, 150, 151).

The hemodynamic efficacy of levosimendan was not associated with an increase in myocardial oxygen consumption. This was shown in a study utilising dynamic positron emission tomography (PET) with ^{11}C-acetate (31). Levosimendan was given as a loading dose of 18 µg/kg followed by an infusion of 0.3 µg/kg/min for 2 hours. Dobutamine and nitroprusside were used as comparators. Levosimendan caused only slight and non-significant increases in myocardial oxygen consumption (+12%), whereas dobutamine significantly increased oxygen consumption (+58%). The effects of nitroprusside were neutral.

Clinical effects in patients

Levosimendan has been studied in more than one thousand patients. The results of the four largest trials (Table 4) and some smaller studies are described in the following chapters.

Table 4. Pivotal studies with levosimendan.

Study (ref.)	Number of patients	Number of LS patients	Comparator	Diagnosis	NYHA Class
Dose-finding (153)	151	95	Placebo/ Dobutamine	CHF	III
Dose-escalation (154)	146	98	Placebo	CHF	III-IV
Active comparator (36)	203	103	Dobutamine	CHF	(III)-IV
Post-AMI (37)	504	402	Placebo	Post-AMI	III-IV
Total	1004	698			

LS = levosimendan
Post-AMI = heart failure after an acute myocardial infarction

Dose-finding study

The therapeutic dose range of levosimendan administered over a 24-hour period was studied in a placebo-controlled, double-blind, parallel-group, randomised study including 151 patients with stable (mainly NYHA class III) heart failure of ischaemic origin (153). Levosimendan was given as a 10-minute loading dose followed by a 24-hour continuous infusion at five doses. The levosimendan doses were 3 µg/kg +

0.05 µg/kg/min (n=16), 6 µg/kg + 0.1 µg/kg/min (n=23), 12 µg/kg + 0.2 µg/kg/min (n=19), 24 µg/kg + 0.4 µg/kg/min (n=23) and 36 µg/kg + 0.6 µg/kg/min (n=14). Dobutamine was administered as a continuous, open-label infusion of 6 µg/kg/min to 20 patients; 21 patients received placebo and 15 vehicle containing ethanol.

When analysed as mean change from baseline, the 24-hour infusion of levosimendan produced significant, dose-dependent increases in cardiac output (Figure 3), stroke volume, and heart rate, and decreases in PCWP (Figure 4), mean blood pressure, mean pulmonary artery pressure, mean right atrial pressure and total peripheral resistance. The relationship between dose and effect was significant for all of these hemodynamic variables. All doses of levosimendan produced significantly larger decreases in PCWP than dobutamine did, and infusions of 0.4 and 0.6 µg/kg/min produced significantly larger increases in cardiac output than dobutamine did. The increase in cardiac output was due partly to an increase in heart rate, especially with higher doses of levosimendan. The hemodynamic effects of levosimendan, especially the decrease in PCWP, tended to increase with time, whereas the effect of dobutamine became attenuated.

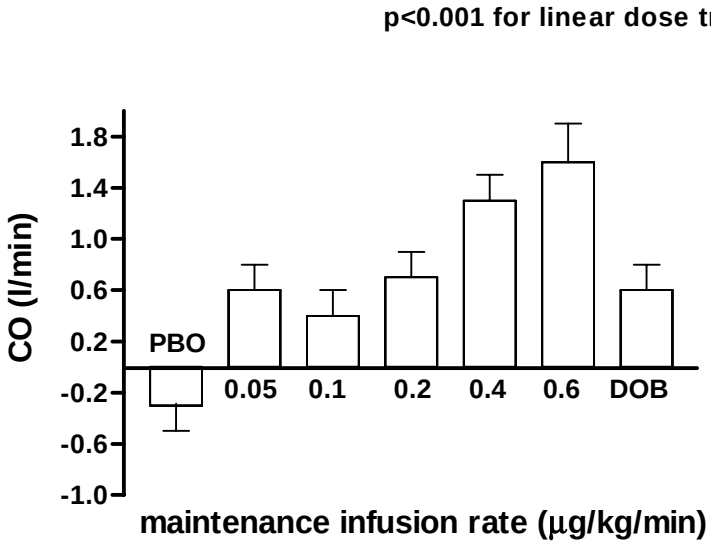

Figure 3. Change in cardiac output at 24 hours compared with baseline after a 24-hour infusion of 5 doses of levosimendan, placebo (PBO), or dobutamine (DOB) in 151 patients with stable heart failure. Mean values are shown. P<0.001 for linear dose trend (153).

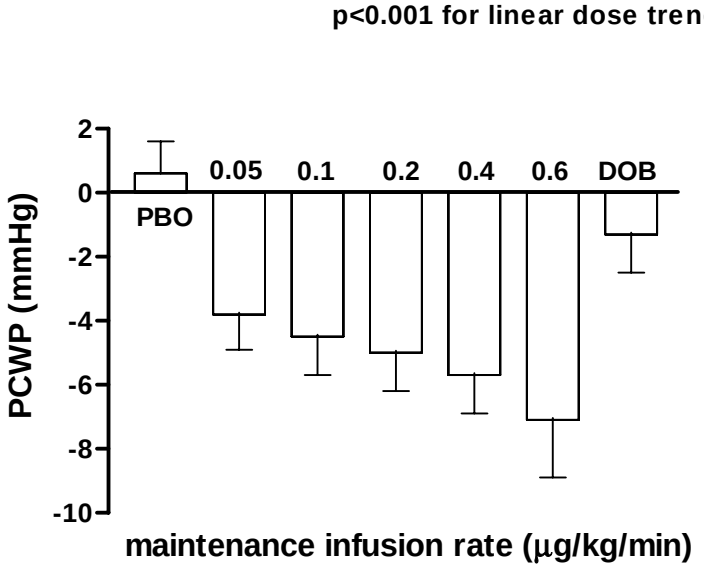

Figure 4. Change in pulmonary capillary wedge pressure at 24 hours compared with baseline after a 24-hour infusion of 5 doses of levosimendan, placebo (PBO), or dobutamine (DOB) in 151 patients with stable heart failure. Mean values are shown. P<0.001 for linear dose trend (153).

The two highest doses of levosimendan (0.4 and 0.6 µg/kg/min) were associated with an increase in the incidence of adverse events, such as sinus tachycardia, hypotension, and a prolongation of the QTc interval.

Dose-escalation study

The second large study with levosimendan evaluated the effects of escalating levosimendan doses on hemodynamics and symptoms in more severely ill heart failure patients in a placebo-controlled, double-blind, parallel-group, randomised study in 146 patients hospitalised for decompensated heart failure (NYHA class III or IV) due to coronary artery disease or dilated cardiomyopathy. The initial phase of the study compared the effects of a 6-hour infusion of levosimendan and placebo (154). Levosimendan (n=98) or placebo (n=48) was initiated with a bolus of 6 µg/kg followed by a continuous infusion of 0.1 µg/kg/min for 1 hour. Thereafter, at hourly intervals a 6 µg/kg bolus was administered and the continuous infusion rate was increased by increments of 0.1 µg/kg/min until a maximum infusion rate of 0.4 µg/kg/min was achieved or a dose-limiting event occurred.

At 6 hours, levosimendan increased stroke volume by 28% (Figure 5) and cardiac index by 39%, while PCWP decreased by 22% (Figure 6) (p<0.001 for both). Heart rate increased by 6 bpm (p<0.001). Levosimendan significantly decreased pulmonary artery pressure, pulmonary vascular resistance, mean arterial pressure, and systemic vascular resistance.

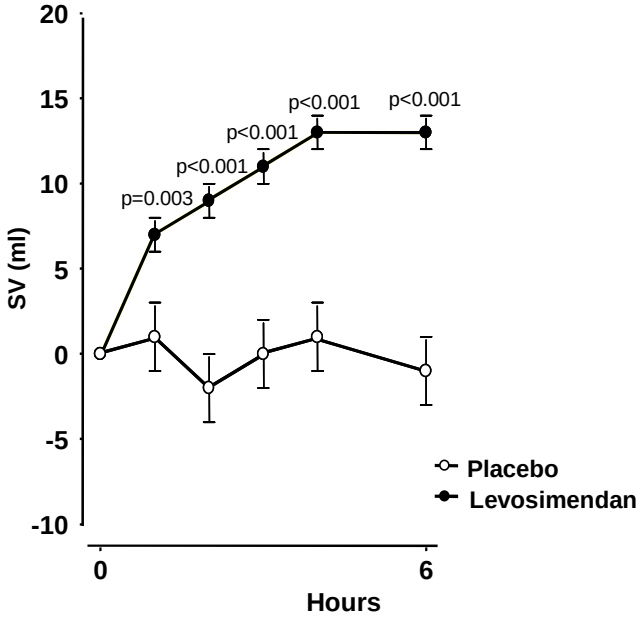

Figure 5. Change in stroke volume compared with baseline after a 6-hour infusion of levosimendan (up-titrated during the first 4 hours) or placebo in 146 patients with NYHA III-IV heart failure. Mean values and SEM are given. P-values denote the difference between levosimendan and placebo at the various time points (154).

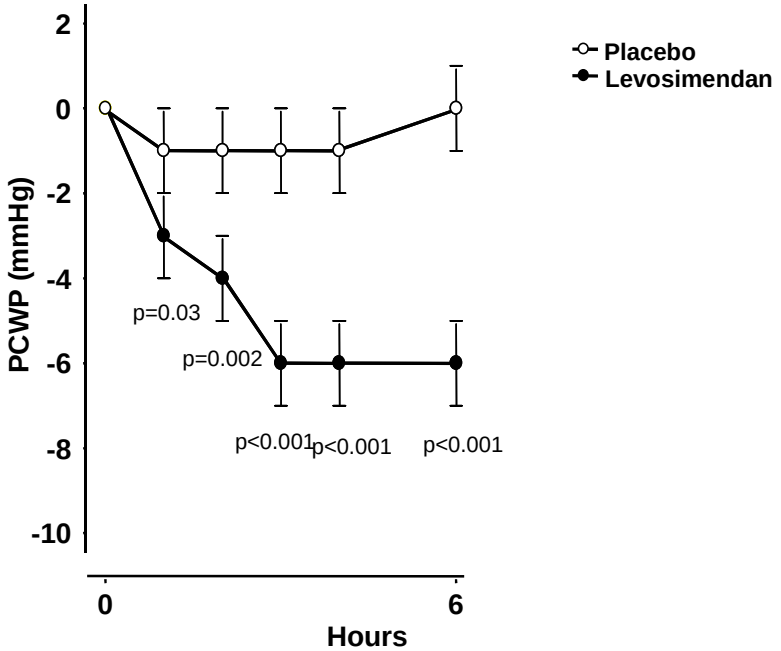

Figure 6. Change in pulmonary capillary wedge pressure compared with baseline after a 6-hour infusion of levosimendan (up-titrated during the first 4 hours) or placebo in 146 patients with NYHA III-IV heart failure. Mean values and SEM are given. P-values denote the difference between levosimendan and placebo at the various time points (154).

The hemodynamic benefits were accompanied by an improvement in the two major symptoms of heart failure: dyspnoea and fatigue. In the intention-to-treat population, more patients reported improvement in dyspnoea at 6 hours in the levosimendan group than in the placebo group (29% versus 15%; p=0.037). There was a trend toward improvement also in fatigue, with more patients in the levosimendan group reporting improvement (42 vs. 22 %; p=0.057).

Comparison to dobutamine

Levosimendan was compared to dobutamine in a double-blind, parallel-group, randomised trial in 203 patients with low-output heart failure who required right heart catheterisation and treatment with an intravenous inotropic drug (the LIDO study) (36). Patients had either an ischaemic or non-ischaemic aetiology of heart failure.

Treatment with levosimendan (n=103) was started with a loading dose of 24 µg/kg infused over 10 minutes and followed by a continuous infusion of 0.1 µg/kg/min. Dobutamine (n=100) was given as a continuous infusion of 5 µg/kg/min without a loading dose. For patients in whom the cardiac index had not increased by at least 30% compared with the baseline value at 2 hours, the dose was doubled: 0.2 µg/kg/min for levosimendan (n=69) and 10 µg/kg/min for dobutamine (n=40). Both infusions were then continued for a total infusion time of 24 hours.

Levosimendan produced significantly greater increases in cardiac output (1.1 versus 0.8 l/min, p=0.048) and significantly greater decreases in PCWP (–7 versus –3 mmHg, p=0.003) than dobutamine at the end of the 24-hour treatment period (Figure 7). Levosimendan also produced greater decreases in pulmonary artery diastolic pressure, mean right atrial pressure, systolic and diastolic blood pressure and total peripheral resistance at 24 hours than dobutamine did. Both levosimendan and dobutamine increased heart rate to a modest and similar degree (6 and 4 bpm, respectively). Dyspnoea improved in 68% and 59% of the patients with baseline symptoms in the levosimendan and dobutamine groups, respectively. Fatigue improved in 63% and 47% with levosimendan and dobutamine, respectively. The differences in symptom improvements were not significant between the two treatments.

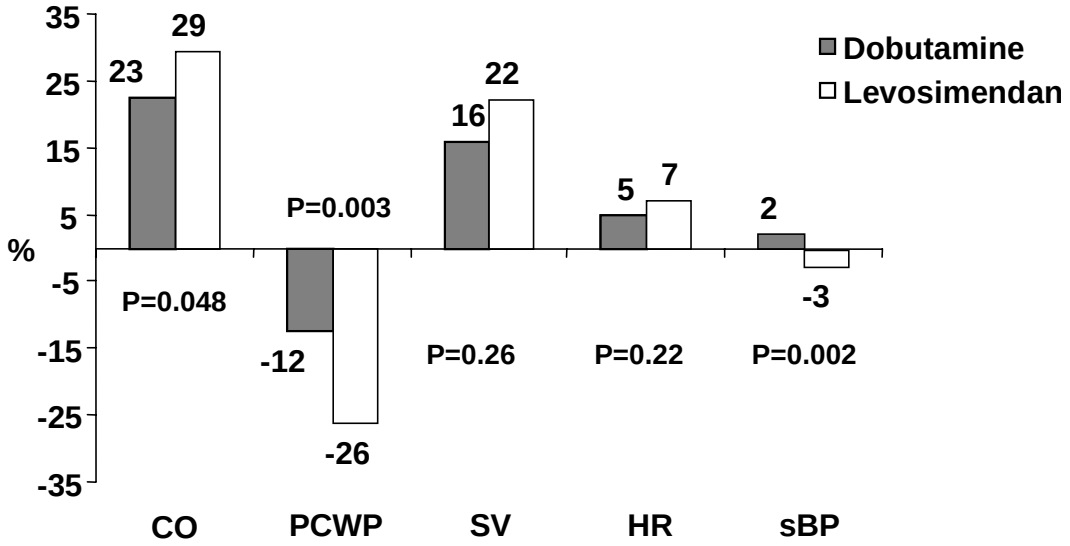

Figure 7. Percent changes in cardiac output (CO), pulmonary capillary wedge pressure (PCWP), stroke volume (SV), heart rate (HR), and systolic blood pressure (sBP) compared with baseline after a 24-hour infusion of levosimendan (n=103) or dobutamine (n=100) in patients hospitalised for acutely deteriorated heart failure (the LIDO study). Mean values are shown. P-values denote the difference between levosimendan and dobutamine (36).

The hemodynamic effects of levosimendan were slightly potentiated by preceding use of a beta-blocker, while the use of a beta-blocker attenuated the effects of dobutamine (Figure 8). Accordingly, the hemodynamic advantages of levosimendan over dobutamine were more pronounced in the presence of previous beta-blockade (p=0.01 for cardiac output and p=0.03 for PCWP). These findings indicate that levosimendan can be used successfully in patients who decompensate while on beta-blockers.

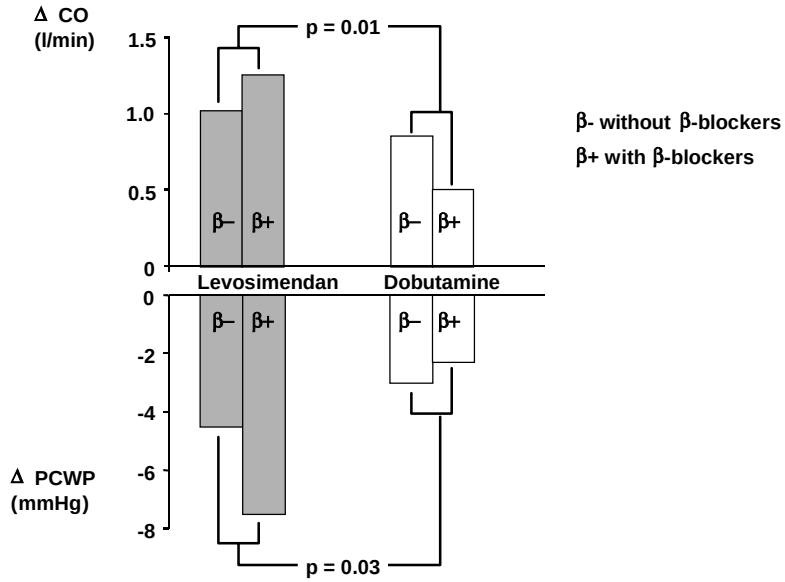

Figure 8. Effects of concomitant use of beta-blockers on cardiac output (CO) and pulmonary capillary wedge pressure (PCWP) after a 24-hour infusion of levosimendan (n=103) or dobutamine (n=100) in patients hospitalised for acutely deteriorated heart failure (the LIDO study). Mean values for the changes at 24 hours compared with baseline are shown. P-values denote the interaction between preceding beta-blocker use and treatment (36).

Mortality was followed as a secondary end-point for 31 days. During that time, 7.8% of patients assigned to levosimendan and 17.0% assigned to dobutamine died (hazard ratio 0.43, p=0.049). The follow-up was retrospectively extended to 180 days and the figures were 26.2% with levosimendan and 38.0% with dobutamine (hazard ratio 0.57, p=0.029) (Figure 9). During the study drug infusion, disturbances of heart rate and rhythm were reported with significantly lower frequency in the levosimendan group than in the dobutamine group (3.9% versus 13.0%, respectively; p=0.023). Angina pectoris, chest pain or myocardial ischaemia was also reported less frequently with levosimendan than with dobutamine (0.0% versus 7.0%, respectively; p=0.013). Headache or migraine (13.6% versus 5.0%; p=0.052) and hypotension (8.7% versus 4.0%; p=0.252) were reported more frequently with levosimendan than with dobutamine.

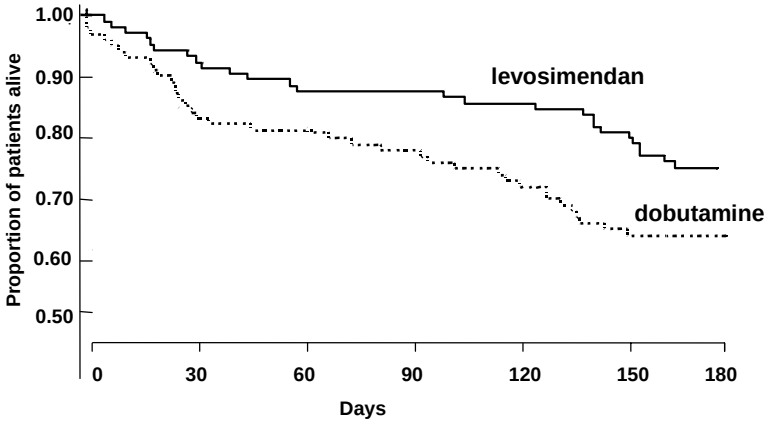

Figure 9. Kaplan-Meier curves showing all-cause mortality during 180 days after initiation of a 24-hour infusion of levosimendan or dobutamine in 203 patients hospitalised for acutely deteriorated heart failure (the LIDO study). Hazard ratio 0.57; 95% confidence interval 0.34 - 0.95, p=0.029) (36).

Safety after an acute myocardial infarction

The safety of levosimendan in patients with left ventricular failure complicating an acute myocardial infarction (AMI) was studied in a placebo-controlled, double-blind, parallel-group, randomised trial in 504 patients within 5 days of AMI (the RUSSLAN study) (37). No invasive hemodynamics were measured in this study. Levosimendan was administered as a 10-minute loading dose followed by a continuous infusion for a total of 6 hours. The following doses were used: 6 µg/kg + 0.1 µg/kg/min (n=103), 12 µg/kg + 0.2 µg/kg/min (n=100), 24 µg/kg + 0.2 µg/kg/min (n=99), 24 µg/kg + 0.4 µg/kg/min (n=100) and similar placebo infusion (n=102).

As the study was concentrating on safety, the primary end-point was the proportion of patients developing clinically significant hypotension and/or myocardial ischaemia as assessed by an independent safety committee. When all four levosimendan groups were combined, the proportions of patients who experienced ischaemia and/or hypotension in the placebo and levosimendan groups were similar (10.8% versus 13.4%, respectively) (p=0.456). Although there was a weak relation between the dose of levosimendan and the risk of hypotension and/or ischaemia (p=0.054), this was fully attributable to the higher frequency observed with the highest dose (19% compared with 11-12 % in the other levosimendan and placebo groups). The combined risk of death and worsening heart failure was significantly lower in patients treated with levosimendan than in patients treated with placebo, both during the 6-hour infusion period (2.0% versus 5.9%, respectively; p=0.033), and at 24 hours (4.0% versus 8.8%, respectively; p=0.044).

All-cause mortality during the 14-day follow-up period (a pre-specified secondary end-point) was significantly lower in levosimendan- than in placebo-treated patients (11.7% versus 19.6%, respectively; p=0.031). The positive effect on mortality was maintained in a retrospective 180-day follow-up (22.6% versus 31.4%, respectively; p=0.053) (Figure 10). It is evident, however, that the reduction in mortality attributable to levosimendan was achieved during the first 14 days. After 14 days the Kaplan-Meier curves remain parallel, thus indicating no additional survival benefit

after that time. There was no relationship between the dose of levosimendan and all-cause mortality.

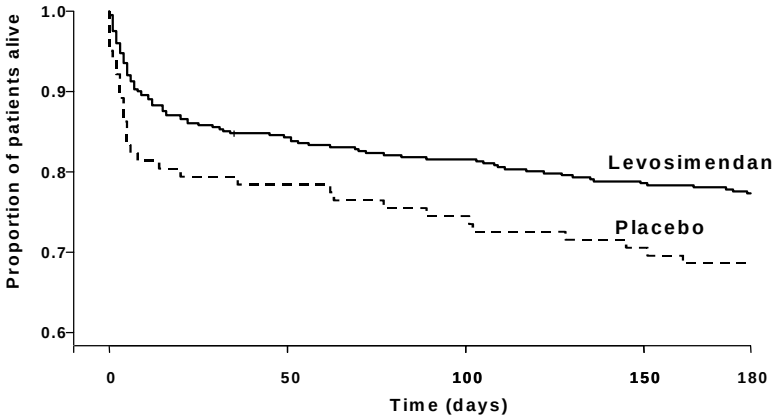

Figure 10. Kaplan-Meier curves showing all-cause mortality during 180 days after a 6-hour infusion of levosimendan or placebo in 504 patients with heart failure complicating an acute myocardial infarction (the RUSSLAN study). Hazard ratio 0.67; 95% confidence interval 0.45 - 1.00, p=0.053) (37).

Other studies

The beneficial effects of levosimendan on hemodynamics are not achieved by an increase in myocardial energy consumption. This was evidenced by utilising dynamic PET technique in 8 hospitalised patients with heart failure (NYHA III-IV) (32). The patients were given levosimendan 18 µg/kg as a loading dose followed by a continuous infusion of 0.3 µg/kg/min for about 5 hours, and placebo in a cross-over fashion. Despite increases in both cardiac output and stroke volume, myocardial oxygen consumption was unaltered by levosimendan.

Levosimendan possesses also anti-stunning effects. This was shown in a randomised, double-blind study in patients with an acute myocardial infarction who had underwent percutaneous transluminal coronary angioplasty (PTCA) (155). The patients received levosimendan 24 µg/kg as a bolus dose (n=16) or corresponding placebo (n=8) 10 minutes after completion of the successful PTCA. The study showed that levosimendan clearly improved the function of stunned myocardium, as shown by a substantial reduction in the number of hypokinetic segments in the left ventricular wall (-2.4) compared with placebo (increase of 0.8) (p=0.016).

The same study also showed that diastolic function was not worsened by levosimendan; end-diastolic pressure-volume ratio and chamber compliance during late diastole changed similarly with levosimendan and placebo. In addition, the index of isovolumic relaxation Tau was improved in the levosimendan group and impaired in the placebo group, which suggests improved diastolic function. A similar finding was observed in a study using intracoronary infusions (156). Ten patients with heart failure received two intracoronary doses without systemic effects (3.75 and 12.5 µg/min) and dextrose (control) as bolus doses. In this study Tau was improved with the higher dose, but was unaffected with the lower dose. In the intracoronary study levosimendan also increased left ventricular +dP/dt dose-dependently at various paced heart rates indicating a direct contractility enhancing effect with levosimendan.

The effects of levosimendan on conduction intervals have been modest. In the above dose-finding study (153), there were no changes in PQ or QRS intervals. The QTc interval (by Bazett equation) was not significantly prolonged at doses of 0.05 - 0.2 µg/kg/min compared with placebo. However, the dose of 0.4 µg/kg/min of levosimendan (two times higher than the recommended dose), which increased heart rate by 10 bpm, also prolonged QTc interval by 15 ms. With the levosimendan dose of 0.6 µg/kg/min (three times higher than the recommended dose), which increased heart rate by 20 bpm, the QTc interval was significantly prolonged by 45 ms. Among other factors, QT interval is dependent on heart rate. Although the Bazett equation is the most commonly used formula for correction of the QT interval, it is inappropriate at high heart rates because it overestimates the QTc interval at faster rates (157, 158). This may well explain the prolongation of the QTc interval observed at high doses of levosimendan.

The confounding effects of changes in heart rate on the QTc interval can be overcome by studying the effects on the uncorrected QT interval at stable heart rates. An intracardiac electrophysiology study was conducted in 10 patients with normal cardiac function who were evaluated for rhythm disorders. Heart rate was kept constant by atrial pacing at various cycle lengths, corresponding to heart rates of 75 to 120 bpm (159). Levosimendan was given as a loading dose of 18 µg/kg followed by a continuous infusion of 0.4 µg/kg/min for a total duration of 30-40 minutes. The QT interval was unchanged compared with baseline at all studied cycle lengths. This indicates that levosimendan does not affect the duration of ventricular repolarisation.

A meta-analysis of ambulatory ECG recordings was done on a total of 792 recordings pooled from 10 studies, which included data from 386 patients. The recordings represented a total duration of >14 000 hours. There were no significant differences between levosimendan and placebo in the occurrence of bradyarrhythmias, atrio-ventricular block or supraventricular or ventricular arrhythmias. Ventricular fibrillation occurred in one patient in the placebo group, while none was seen with levosimendan. No cases of Torsades de Pointes tachycardia were found in any treatment groups. New occurrence of supraventricular or ventricular tachycardia or other proarrhythmia according to Morganroth was similar during levosimendan and placebo administration (34).

Summary of the main hemodynamic effects of levosimendan

The most important hemodynamic effects of levosimendan are summarised in Table 5 (36, 153, 154).

Table 5. Hemodynamic effects of levosimendan

Variable
Pulmonary capillary wedge pressure ↓↓↓
Cardiac output (index) ↑↑
Stroke volume↑
Systemic vascular resistance ↓↓
Pulmonary vascular resistance ↓↓
Heart rate ↑
Systolic blood pressure ↓
Diastolic blood pressure ↓

↓ = decrease, ↑ = increase

Pharmacokinetics

Levosimendan is mainly eliminated by metabolism. The metabolic pathways in experimental animals and in man are similar. The main pathway is conjugation with glutathione to inactive metabolites and the minor pathway (only approximately 5% of the total levosimendan dose) is reduction in the intestine to the aminophenylpyridazinone metabolite OR-1855. The amine metabolite is further metabolized by acetylation to the N-acetylated conjugate OR-1896 (160). Also an inactive hydration product, OR-1420, is formed through addition of water to one of the nitrile groups. Levosimendan is excreted via urine and faeces as conjugates and only traces of unchanged levosimendan are found in urine or faeces in experimental animals and in man (38, 161). Levosimendan metabolism is described in Figure 11.

The metabolite OR-1896 has been shown to have hemodynamic properties similar to those of the parent drug (148, 149, 162). In different experimental animals, the elimination half-life of levosimendan was 0.25 to 1.3 hours (160), whereas that of the metabolite OR-1896 was 4.7 to 6.5 hours (unpublished results, Vuorela A and Kurkijärvi U).

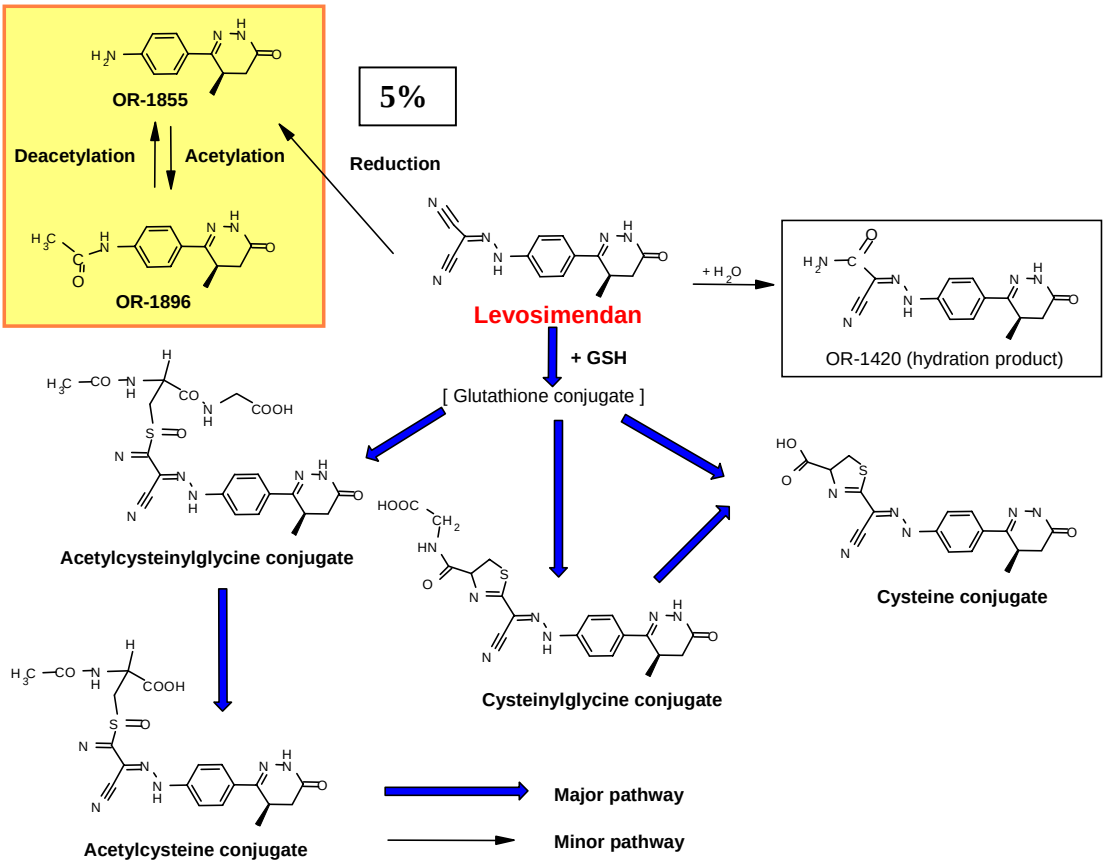

Figure 11. Metabolism of levosimendan.

The terminal elimination half-life ($t_{1/2el}$) of levosimendan is about 1 hour in both healthy volunteers and patients with heart failure (38-40). Levosimendan is highly bound to plasma proteins (97-98%) (38, 163). Total plasma clearance of levosimendan is approximately 200-360 ml/min (38-40) with no significant difference between patients with congestive heart failure and healthy volunteers (38). The concentrations of levosimendan increase dose proportionally (164). The bioavailability of levosimendan from an oral solution is approximately 85% in both patients with heart failure and healthy volunteers (38).

Several interaction studies with levosimendan have been performed. Cytochrome-P-450-enzymes seem not to be involved in the metabolism of levosimendan as indicated by the lack of pharmacokinetic or hemodynamic interactions with itraconazole, warfarin or felodipine (163, 165, 166) Furthermore, studies with captopril, isosorbide-5-mononitrate and carvedilol have revealed no relevant hemodynamic or pharmacokinetic interactions (152, 167, 168)

A single dose study with intravenous [^{14}C] levosimendan showed that the elimination half-life of the total drug (unchanged levosimendan and the metabolites) was considerably longer than that of levosimendan (38). At that time, the reduction metabolites OR-1855 and OR-1896 were not known but the results suggested that metabolites with longer elimination half-lives than that of the parent drug are probably formed after levosimendan administration. Later, the metabolites were detected in preclinical studies. The metabolite OR-1855 was first detected in man in a

cross-over study in which levosimendan was administered as 2-mg single doses intravenously and orally to healthy volunteers. The metabolite OR-1855 was detected in plasma only long after the parent drug levosimendan had disappeared from the plasma (40). Although already then preclinical data suggested OR-1896 to be an active metabolite, this metabolite could not be measured in the study as there was a validated method for analysing only the metabolite OR-1855 at that time. Later, the plasma concentrations of the metabolites OR-1855 and OR-1896 were measured in the multicenter studies presented earlier (36, 153, 154). The concentrations of the metabolites were seen to be increasing even after stopping levosimendan infusion but the follow-up in these studies was too short to draw any reliable conclusions on the pharmacokinetics of the metabolites.

3. AIMS OF THE STUDY

Levosimendan is a new compound for the treatment of heart failure. The pharmacokinetics of the parent drug, levosimendan, had only been studied after bolus doses and no information on the effects of extended levosimendan infusions was available. Furthermore, the pharmacokinetics and hemodynamic effects of the metabolites of levosimendan had not been studied in man.

The aims of the present thesis can be summarised as follows:

1. To investigate the role of levosimendan´s circulating metabolites OR-1855 and OR-1896 in the hemodynamic responses achieved by levosimendan infusion
2. To investigate the pharmacokinetics of the parent compound levosimendan in patients with severe heart failure, and when administered as extended infusions
3. To investigate the pharmacokinetics of the metabolites OR-1855 and OR-1896
4. To investigate the tolerability of levosimendan when administered intravenously for up to 7 days

4. SUBJECTS AND METHODS

The four publications included in this thesis are based on three different studies. In the following chapters the four publications are called studies I, II, III A and III B. The studies can be summarised as follows:

Study I (publication I)

- presents the results of 98 levosimendan-treated patients in a US multicentre study
- levosimendan was administered for either 24 or 48 hours

Study II (publication II)

- a pharmacokinetic/-dynamic study in 12 patients
- levosimendan was administered for 24 hours

Study III A and B (publications III and IV)

- a pharmacokinetic/-dynamic study in 24 patients
- levosimendan was administered for 7 days at two different infusion rates
- Study III A concentrates on the hemodynamic results
- Study III B concentrates on the pharmacokinetic results

4.1 Study subjects

A total of 134 subjects were included in the studies presented in this thesis. All patients had severe chronic heart failure with NYHA III-IV symptoms. The etiology of heart failure was ischemic, dilative, hypertensive or a combination of these. Study I was performed in 20 US study sites with originally 146 patients (154). Study I presents the results of the 98 patients who received levosimendan in that study. Studies II and III (A and B) were performed in Finland and Estonia with a total of 19 and 17 patients in each country, respectively.

The studies had specific inclusion and exclusion criteria, which the subjects had to fulfill before decision of the entry to the study was made. The main inclusion criteria for study I were: adult (> 21 years) male or female patients with stable or unstable chronic congestive NYHA III-IV heart failure and left ventricular ejection fraction under 30%. Additionally the patient had to fulfil the following invasive hemodynamic criteria: baseline value of PCWP of ≥15 mmHg and CI <2.5 L/min/m^2. The main exclusion criteria were angina-limited exercise, unstable angina or acute myocardial

infarction within the previous 8 weeks, obstructive cardiomyopathy, uncorrected primary stenotic valve, history of ventricular flutter or fibrillation or symptomatic ventricular tachycardia, symptomatic primary pulmonary disease, supine systolic blood pressure <85 mm Hg or >200 mm Hg, resting heart rate >115 bpm, serum creatinine >225 μmol/l, liver transaminases > two times upper limit of normal, uncorrected hypokalemia or hyperkalemia (serum potassium <3.5 mmol/l or >5.5 mmol/l) or treatment with another investigational therapy in the 30 days prior to study inclusion.

The main inclusion and exclusion criteria for studies II and III (A and B) were almost the same and can be summarised as follows: adult (> 18 years) male or female patients with chronic congestive NYHA III-IV heart failure and left ventricular ejection fraction under 40% were included. The main exclusion criteria were: supine systolic blood pressure <95 mmHg, supine heart rate <50 or >120 bpm, serum potassium <3.5 mmol/l or >5.5 mmol/l, serum creatinine >300 μmol/l, blood hemoglobin <110 g/l, recent cardiovascular event (e.g. unstable angina, myocardial infarction, coronary revascularization procedure, stroke, transient cerebral ischemic attack or life-threatening ventricular arrhythmias). Additionally, the following medications were not allowed for two weeks before the study: antiarrhythmic drugs (except amiodarone and beta-blockers); positive inotropic drugs (except digoxin) and pressor agents used specifically for hypotension.

4.2 Study designs

Study I was a multicentre trial in 20 US centers with 3 separate phases. Patients were recruited between June 1995 and May 1997. The design of the study is described in Figure 12. In the double-blind, placebo-controlled Phase 1, 98 patients received escalated doses of intravenous levosimendan and 48 patients corresponding placebo for 6 hours. The results of this phase of the trial have been published previously by Slawsky (154). Phase 2 started at 6 hours. The patients on levosimendan continued to receive levosimendan up to 24 hours as an open-label infusion. Phase 3 started at 24 hours. The patients were randomized (1:1 ratio) to continue on levosimendan (levosimendan continuation group) or placebo (levosimendan withdrawal group) double-blindly for up to 48 hours. During phase 1, levosimendan treatment was initiated with a bolus of 6 μg/kg followed by a continuous infusion of 0.1 μg/kg/min for 1 hour. Thereafter, at hourly intervals, a new 6 μg/kg bolus and a continuous infusion increased by increments of 0.1 μg/kg/min was administered till a maximum of 0.4 μg/kg/min infusion rate was achieved or a dose-limiting event occurred. At the beginning of phase 2 at 6 hours, the achieved levosimendan infusion rate was halved and continued at that rate till 24 hours. At the beginning of phase 3 at 24 hours, the patients who were randomized to continue receiving levosimendan, continued to receive the drug at the same rate as during phase 2. At any phase of the study, the study drug was discontinued temporarily or permanently if any of the following dose-limiting events occurred: 1) heart rate > 130 bpm or an increase in heart rate > 15 bpm above baseline for 10 minutes, 2) symptomatic hypotension or a drop in systolic blood pressure to < 75 mmHg, 3) decrease in PCWP to ≤ 10 mmHg or 4) any adverse event that, in the opinion of the site investigator, required dose modification. If a dose-

limiting event occurred, the study drug was discontinued until the event resolved and was then restarted at the next lower dose.

The hemodynamic follow-up was up to 48 hours after initial start of the study drug, pharmacokinetic samples were taken up to 54 hours and serious adverse events including mortality were followed up to 14 days.

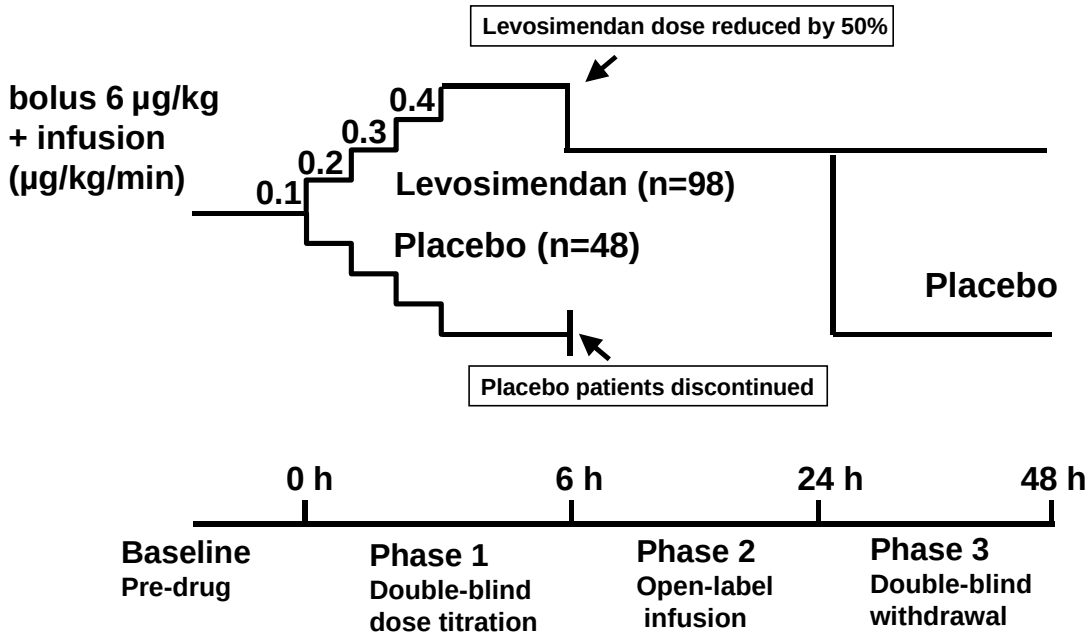

Figure 12. Design of study I.

Studies II and III (A and B) had non-randomised, open-label design. They were performed in two study centers: Helsinki University Central Hospital, Helsinki, Finland and Mustamäe Hospital, Tallinn, Estonia. The recruitment of patients in study II took place between May 1999 and September 1999 and in study III between September 1998 and May 1999.

In study II, the 12 patients were initially hospitalised for 5 days: baseline hemodynamics were assessed during the first day, 24-hour continuous levosimendan infusion was given on the second day, and patients were followed in hospital for an additional three days. The dose of levosimendan was 0.2 µg/kg/min for 24 hours for all patients. During the 2-week follow-up the patients came to the hospital daily for ECG recordings and blood sampling only. Six of the 12 patients were hospitalised for 24 hours one week after stopping the infusion and all patients also for 24 hours at the end of the 2-week follow-up period for Holter recordings (Figure 13).

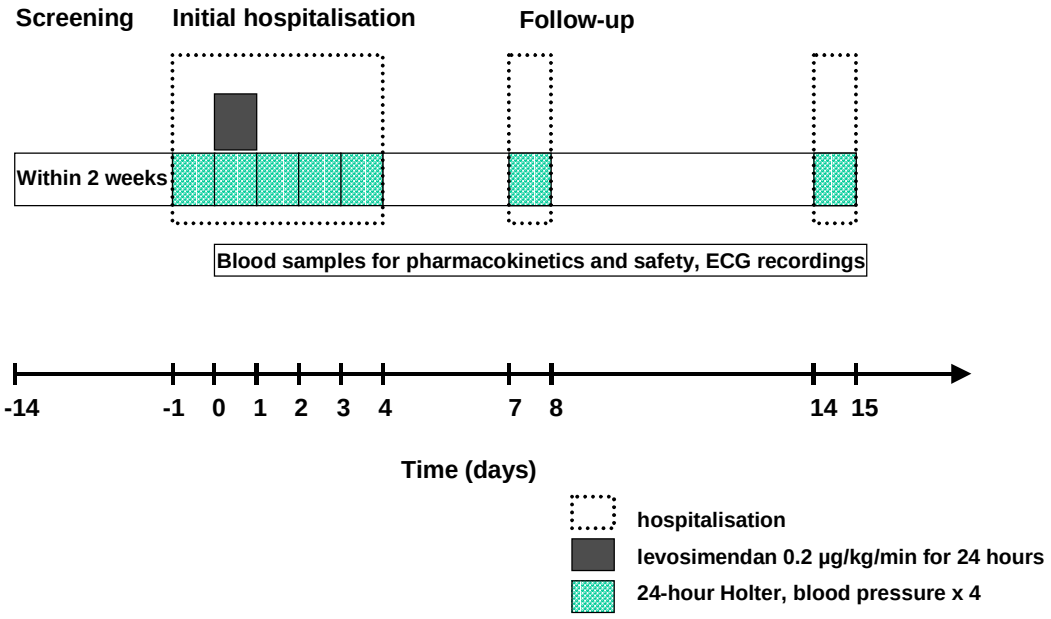

Figure 13. Design of study II.

Study III consisted of a 7-day continuous infusion of levosimendan and a follow-up of 10 to 15 days thereafter. Twelve patients received levosimendan at a continuous infusion rate of 0.05 µg/kg/min for 7 days and twelve at a rate of 0.1 µg/kg/min for 7 days. The follow-up period was 10 days for six of the 12 patients receiving the higher dose and 13 to 15 days for all the other patients. The patients were hospitalised during the infusion and for two days thereafter. During the rest of the follow-up, they came daily at about 0900 hours to the hospital for clinical measurements and pharmacokinetic blood sampling (Figure 14).

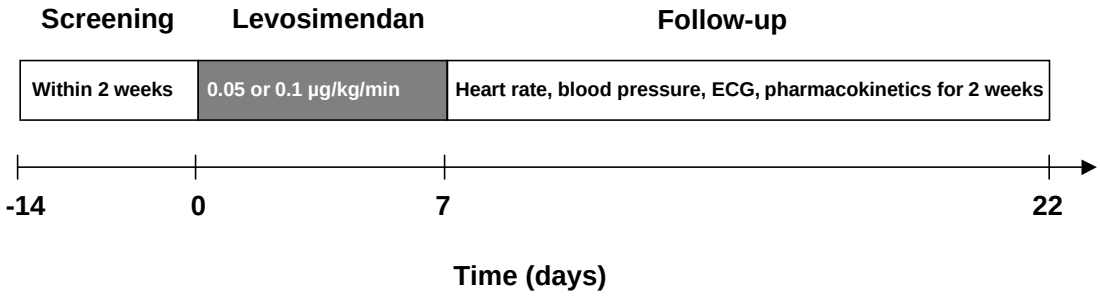

Figure 14. Design of study III.

4.3 Hemodynamic measurements

Study I

At least 2 hours following insertion of a pulmonary artery catheter, two sets of hemodynamic measurements, separated by 10 minutes, were averaged and used as baseline hemodynamic values. Measurements included: pulmonary capillary wedge pressure, pulmonary artery pressure, right atrial pressure and cardiac output (by thermodilution method). Heart rate was determined from the ECG, and blood pressure was determined by arm cuff or intra-arterial monitor. Stroke volume, systemic vascular resistance and pulmonary vascular resistance were calculated using standard equations. Hemodynamic measurements were obtained at baseline, repeatedly during the first 6 hours, at 23.5 and 24 hours, and at 25, 26, 28, 30, 47.5 and 48 hours. The measurements at 5.5 and 6 hours, 23.5 and 24 hours and 47.5 and 48 hours were averaged to represent the values at 6, 24 and 48 hours, respectively.

Study II

Individual 24-hour mean heart rate was determined from Holter recordings that were taken during the day before the study drug infusion (baseline), during the infusion day, on each of the following three days after stopping the infusion and at the end of the 2-week follow-up. An additional Holter recording was taken one week after stopping the study drug infusion in six of the patients. To stabilize the conditions for Holter recordings, the patients were hospitalised for all of the recordings. The patients were, however, allowed to be out of bed according to the best of their ability during the recordings, and also during the study drug infusion. The Holter recordings were measured using Holter Recorder Marquette (Series 8500, Marquette Electronics, Inc., Milwaukee, WI, USA). Four of the planned 78 24-hour Holter recordings were technically inadmissible and they were omitted from the mean calculations.

Blood pressure was measured four times daily during waking hours (about every 4 hours) on the same days as the Holter recordings were taken. The measurements were performed in supine position after a rest of at least 10 minutes either manually by auscultatory method with a mercury sphygmomanometer or by an automatic oscillometric device (Omron M4, model HEM-722C, Omron Matsusaka Co, Japan). In every case, one measurement was performed. If the measurement failed, it was repeated after two minutes.

Study III A

A 12-lead ECG was registered for the determination of heart rate before the infusion, on the 1st, 3rd, 5th, and 7th day of the infusion, at the end of the infusion and on the 1st, 2nd, 3rd, 7th and 10th day during the follow-up period. An additional 12-lead ECG was registered once during the follow-up days 13, 14 or 15 in all patients receiving the dose 0.05 µg/kg/min and in 6 patients receiving the dose 0.1 µg/kg/min of levosimendan. On the remaining study days, heart rate was recorded from either limb lead ECGs or by palpation from the radial artery. The patient rested in supine position for at least 10 to 15 minutes before each heart rate measurement. The ECG recordings were performed at a paper speed of 50 mm/s (Siemens Sicard 440/740, Siemens-Elema Inc., USA and Schiller CS-100, Schiller AG, Switzerland).

Blood pressure was measured daily at the same time as the heart rate. The measurements were performed in supine position after a rest of at least 10-15 minutes either manually by auscultatory method with a mercury sphygmomanometer or by an automatic oscillometric device (Omron M4, model HEM-722C, Omron Matsusaka Co, Japan). In every case, only one measurement was performed. If the measurement failed, it was repeated after two minutes.

There were two patients, one in each group, with a pacemaker and a non-variable heart rate. These patients were excluded from the mean heart rate calculations but were included in the mean blood pressure calculations.

4.4 Symptom assessment

Symptoms were only assessed in study I. Specific inquiries were made about the presence and severity of dyspnoea and fatigue, the two major symptoms of heart failure. These symptoms were evaluated by both the patient and the physician at baseline, at 6 hours, at 24 hours and at 48 hours. Patients were asked to grade their symptoms according to a 5-point scale with 1 denoting the most severe symptoms (extreme dyspnoea or fatigue) and 5 denoting no symptoms.

4.5 Pharmacokinetics

Blood samples

Study I

Levosimendan plasma concentrations were determined from venous blood samples (5 ml) drawn within 10 minutes of the start of the infusion, repeatedly during first 6 hours, repeatedly between 24 and 30 hours, and at 48 and 54 hours. An additional 5-ml blood sample was collected for the measurement of plasma levels of the metabolites OR-1855 and OR-1896 at the following time-points: within 10 minutes of the start of the infusion and at 24, 30, 48 and 54 hours. Blood samples were drawn from the antecubital vein contralateral to the one that the study drug was infused in.

Study II

Venous blood samples (3 ml) for the determination of concentrations of levosimendan and its metabolites OR-1855 and OR-1896 were drawn immediately before levosimendan infusion (0-hours) and 2, 4, 8, 12 and 24, 24.5, 25, 26, 28, 36, 48 and 60 hours after starting the infusion. Thereafter, samples were drawn daily every morning (every 24 hours) until 10 days of the follow-up. The last sample was drawn during the follow-up days 13, 14 or 15. During the study drug infusion, blood samples were drawn from the antecubital vein contralateral to the one that the study drug was infused in.

Study III B

Venous blood samples were drawn similarly as in study II. The time-points were as follows: immediately before levosimendan infusion (0-hours) and 2, 4, 8, and 12

hours after starting the infusion on the first study day. Thereafter, blood samples were drawn once daily in the mornings during the 7-day infusion. After stopping the infusion, samples were drawn at 0 minutes and at 0.5, 1, 2, 4, 12, 24 and 36 hours. Thereafter, samples were drawn daily at 0900 hours (every 24 hours) until day 10 of the follow-up. One extra blood sample was also drawn 3 to 5 days after the last sample (13 to 15 days after stopping the infusion) for the determination of metabolite OR-1855 and OR-1896 concentrations from all patients receiving the dose 0.05 µg/kg/min and from 6 patients receiving the dose 0.1 µg/kg/min of levosimendan. During the study drug infusion, blood samples were drawn from the antecubital vein contralateral to the one that the study drug was infused in.

Urine samples

Urine samples were collected only in study III B from patients who received levosimendan at a dose of 0.05 µg/kg/min for 7 days. The samples were collected in fractions of 0-6 h and 6-24 h after stopping the infusion. The volume of each fraction was measured and aliquots (2 x 10 ml) were stored for quantitative analysis.

Pharmacokinetic analyses

The most comprehensive analyses were performed in study III B and they are described below in detail.

Study III B

Human plasma and urine samples were analyzed for concentrations of levosimendan and its metabolites OR-1855 and OR-1896. Protein binding of levosimendan, OR-1855 and OR-1896 *in vivo* was studied using the ultrasentrifugation technique. Plasma samples were ultrasentrifuged after which the concentration of levosimendan, OR-1855 and OR-1896 was determined in the ultrasentrifugation supernatant.

Blood was centrifuged within 10 minutes of sampling. The plasma was separated and transferred into two polypropylene tubes, frozen immediately and kept at -70°C until analysis. The volume of each urine fraction was measured and 10-ml samples were pipetted into polypropylene tubes, frozen and stored at -70°C until analysis.

All the samples were analysed using a validated high performance liquid chromatographic/tandem mass spectrometric (LC-MS/MS) method. The sample preparation for the determination of levosimendan and its metabolites OR-1855 and OR-1896 in all three biological matrices involved the addition of internal standards and liquid-liquid extraction with a mixture of ethyl acetate and hexane. The liquid chromatographic system consisted of a HP Model 1090 Series II pump and an autosampler (Hewlett Packard, USA). All three liquid chromatographic separations were performed with LiChrosorb RP-18 250 x 4 mm, 10 µm columns (E. Merck). The column effluent was directed through a heated nebulizer interface into a PE Sciex API 300 triple quadrupole mass spectrometer (Perkin-Elmer Sciex Instruments, USA). The mass spectrometer was operated in the multiple reaction monitoring (MRM) mode.

For levosimendan the intra-assay precision values (% CV) ranged from 0.4% to 11% in plasma, from 0.8% to 1.8% in urine and from 0.7% to 2.9% in supernatant of plasma. For OR-1855, the intra-assay precision ranged from 1.8% to 13% in plasma, from 1.7% to 5.0% in urine and from 1.4% to 12% in supernatant of plasma. For OR-

1896, the intra-assay precision ranged from 1.1% to 10% in plasma, from 1.0% to 7.3% in urine and from 2.9% to 5.2% in supernatant of plasma.

The inter-assay precision and accuracy were determined from the analysis of quality control samples. The inter-assay precision in plasma for levosimendan ranged from 1.9% to 8.4%, for OR-1855 from 1.8% to 8.5%, and for OR-1896 from 3.8% to 7.0%. The accuracy values were consistently within ±5% of the target.

The limit of quantitation was 0.2 ng/ml for levosimendan in all three matrices. For OR-1855 the limit of quantitation was 1.0 ng/ml in plasma, 2.0 ng/ml in urine and 0.5 ng/ml in supernatant. For OR-1896 the limit of quantitation was 0.2 ng/ml in plasma and supernatant and 0.5 ng/ml in urine.

Study I

Unlike in studies II and III, in study I levosimendan plasma concentrations were analyzed using an HPLC-UV method (169) and the limit of quantitation for levosimendan was 5.0 ng/ml. The plasma metabolite concentrations were analyzed with two different HPLC-MS/MS systems (PE Sciex API III and PE Sciex API 300) and the methods of the two systems were cross-validated. Within-batch precision of the methods varied from 2.2 to 7.2% for OR-1855 and from 1.7 to 6.8% for OR-1896. The limit of quantitation for OR-1896 was 0.2 ng/ml and for OR-1855 0.5 ng/ml.

Study II

The limits of quantitation for levosimendan and the metabolites in plasma were the same as in study III B. The within-batch precision at the limit of quantitation was 9.1% (CV) or lower for levosimendan, 8.7% or lower for OR-1855 and 9.9% or lower for OR-1896. At the upper limit of quantitation, 40 ng/ml, the within-batch precisions were 2.7% or lower, 3.4% or lower and 1.3% or lower, respectively. The between-batch precisions as determined from the quality control samples were as follows: 8.4% at 0.600 ng/ml and 2.5% at 40.0 ng/ml for levosimendan, 8.5% at 1.00 ng/ml and 4.0% at 40.0 ng/ml for OR-1855, and 7.0% at 0.600 ng/ml and 3.8% at 40.0 ng/ml for OR-1896.

Pharmacokinetic calculations

Study I

No pharmacokinetic calculations were performed in study I. Plasma concentration-time curves were drawn for levosimendan and the metabolites OR-1855 and OR-1896.

Studies II and III B

Pharmacokinetic parameters of levosimendan, OR-1855 and OR-1896 were determined with a computer program by using non-compartmental methods (WinNonlin® Professional, Version 1.5., Pharsight Corp., Mountain View, CA, USA). Maximum concentration (Cmax) and time to maximum concentration (Tmax) for levosimendan, OR-1855 and OR-1896 were registered from the plasma concentration-time data for each subject. Steady-state concentrations of levosimendan were determined only in study III B. The average concentration from 8 hours after starting

the infusion to the end of the infusion (168 h) was calculated. The terminal elimination half-life was calculated from the terminal slope of the concentration-time profiles as follows: $t_{1/2} = \ln(2)/\lambda$, where λ was the elimination rate constant. Area under the curve (AUC) was calculated from the beginning of the 24-hour infusion to the last measurable concentration by the trapezoidal rule. AUC was also extrapolated to infinity using the following equation: $AUC^{0-\infty} = AUC^{0-last} + AUC^{last-\infty}$ (in which $AUC^{last-\infty} = C_{last} / \lambda$)

Clearance (CL_{tot}) and volume of distribution (V_d) were calculated for levosimendan using the following equations: $CL_{tot} = Dose / AUC^{0-\infty}$ and $V_d = Dose / (AUC^{0-\infty} \cdot \lambda)$

Protein binding

The plasma protein binding was studied only in study III B. The percentage of free unbound fraction (fu) of levosimendan, OR-1855 and OR-1896 was calculated as follows: $fu(\%) = C_{supernatant}/C_{plasma} \times 100\%$, where C is the concentration in supernatant or in plasma.

4.6 Safety assessments

Adverse events

Adverse events (AEs) elicited by active inquiry were recorded in all studies. The investigator assessed the frequency, severity and outcome of the AE and causality of the study drug to the AE. In study I, the follow-up was 54 hours after starting the study drug infusions. However, serious adverse events including death, were followed up to 14 days. In studies II and III (A and B) all adverse events, including serious AEs, were followed for up to 2 weeks after stopping the study drug infusion.

Safety laboratory variables

The safety laboratory variables followed in the studies are presented in Table 6. The local laboratories of the study sites analyzed the samples.

Table 6. Safety laboratory variables assessed in the studies.

	Study I	Study II	Study III
Variable	**BL, + 6 h, +24 h, +48 h**	**BL, +24 h, +15 days**	**BL, +7 days, +22 days**
B-haemoglobin	x	x	x
Red blood cell count	x	x	x
B-Haematocrit	x	x	x
B-Reticulocyte count		x	
White blood cell count	x	x	x
White blood cell differential	x		
Platelets	x	x	x
S-Potassium	x	x	x
S-Sodium	x	x	x
S-Chloride	x		
S-Creatinine	x	x	x
B-Glucose	x	x	x
S-Alanine aminotransferase	x	x	x
S-Aspartate aminotransferase	x	x	x
S-Gammaglutamyltransferase	x	x	x
S-Alkaline phosphatase	x	x	x
S-Lactate dehydrogenase	x		
S-Protein	x		
S-Albumin	x	x	x
S-Calcium	x		
S-Uric acid	x		
S-Bilirubin (total)	x	x	
S-Bilirubin (unconjugated)		x	
S-Haptoglobin		x	
S-prothrombin time	x		
S-C-reactive protein		x	x
U-Glucose	x	x	x
U-Protein	x	x	x

BL = baseline, S =serum, B = blood, U = urine

Electrocardiograms

ECGs were taken repeatedly in all studies and the PQ, QRS and QT intervals were measured manually from these. The QTc interval was calculated from the QT interval by the formula of Bazett (170):

$$QTc = QT / \sqrt{RR}$$

Holter monitoring

24-hour ambulatory ECG recordings were performed in study II. Potentially clinically significant arrhythmic events in Holter recordings were determined. For each patient, the day before the infusion was the baseline to which the changes on the other days were compared. The occurrence of ventricular fibrillation, sustained ventricular tachycardia (VT) (duration > 30 seconds, frequency > 120 bpm) and VT runs (duration ≥ 3 beats and < 30 seconds, frequency > 120 bpm) were registered. The Holter recordings were measured using Holter Recorder Marquette (Series 8500, Marquette Electronics, Inc., Milwaukee, WI, USA) and one experienced cardiologist analyzed all the recordings.

4.7 Statistical methods

For quantitative variables, results were summarized using descriptive statistics. All statistical calculations were carried out using SAS software (SAS Institute Inc., Cary, NC, USA). P-values of < 0.05 were considered to be statistically significant.

Study I

Changes in hemodynamics between 6 and 24 hours were evaluated by paired t-test. Changes in hemodynamics between 24 and 48 hours were evaluated using an analysis of variance (ANOVA) model with effects for treatment, center and treatment-by-center interaction. Symptoms of dyspnoea or fatigue were assessed by the Cochran-Mantel-Haenszel test.

Study II

The statistical significance of the time-effect on changes from baseline was tested using model of repeated measurements (proc MIXED). The baseline values were used as covariates. If the overall time-effect was significant, paired t tests for each visit were used for further evaluation.

Study III (A and B)

Analysis of covariance (ANCOVA) for repeated measurements was used to evaluate statistical significances. Baseline values of the variables were used as covariates and included in the statistical model with dose level and time.

4.8 Ethical aspects

The study protocols and informed consent forms were approved by the Ethics Committees or Institutional Review Boards of the study hospitals. Written informed consent was obtained from all patients prior to inclusion in the study. The studies followed the recommendations for biomedical research involving humans found in the Declaration of Helsinki of the World Medical Assembly.

5. RESULTS

5.1 Study profiles

The profile of study I is presented in Figure 15. In each of the three phases of the study some patients were withdrawn from the study drug. The reasons for permanent discontinuations are presented in Table 7. The most frequently seen reason for permanent discontinuation was exaggerated hemodynamic response, i.e. decrease in PCWP to ≤ 10 mmHg either alone or in combination with increase in heart rate. This was most evident in those who were randomized to receive levosimendan throughout the 48-hour study period (levosimendan continuation group).

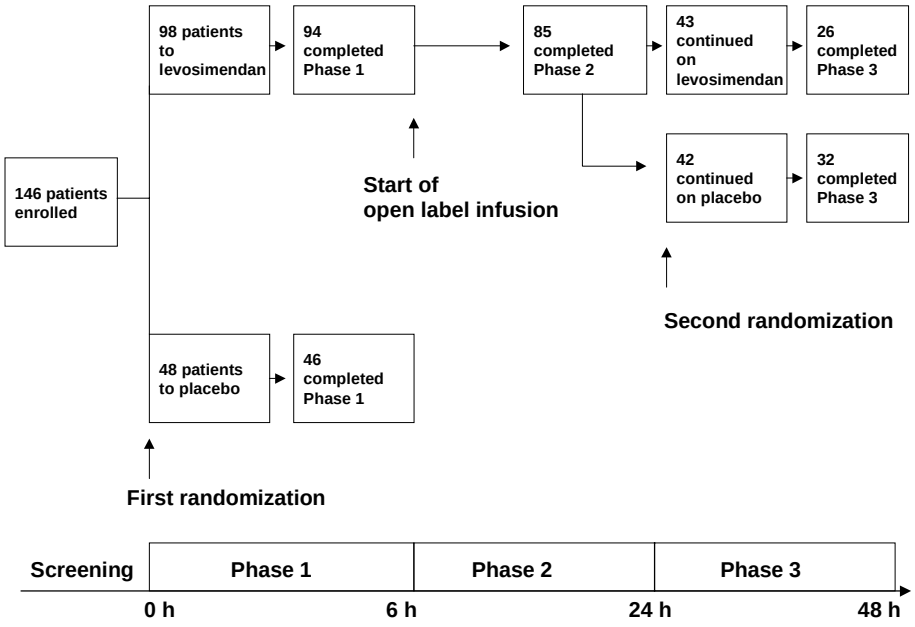

Figure 15. The profile of study I.

Table 7. Reasons for permanent discontinuation of the study drug in individual study subjects during different phases in study I.

Phase 1	**Levosimendan (98)**	**Placebo (48)**
	Lack of efficacy (2)	Lack of efficacy (2)
	HR↑ (1)	
	Ischaemia in ECG (1)	
Phase 2	**Levosimendan (94)**	
	PCWP↓ (3)	
	PCWP↓ + HR↑ (2)	
	HR↑ (2)	
	BP↓ (1)	
	Lack of efficacy (1)	
Phase 3	**LS continuation (42)**	**LS withdrawal (43)**
	PCWP↓ (6)	PCWP↓ + HR↑ (3)
	HR↑ (3)	HR↑ (2)
	Headache (2)	BP↓ (2)
	BP↓ (1)	PCWP↓ (1)
	HR↑ + BP↓ (1)	HR↑ + BP↓ (1)
	Fever + PCWP↓ + HR↑ (1)	Fever + HR↑ (1)
	Sepsis (1)	Lack of efficacy (1)
	Rash (1)	

PCWP↓ = decrease in pulmonary capillary wedge pressure to ≤ 10 mmHg
HR↑ = heart rate > 130 bpm or an increase in heart rate > 15 bpm above baseline for 10 minutes
BP↓ = symptomatic hypotension or a drop in systolic blood pressure to < 75 mmHg
LS = levosimendan

In study II, all 12 patients recruited into the study also completed the study according to protocol.

In study III, originally 26 patients were recruited into the study. One patient was excluded according to the exclusion criteria (life-threatening arrhythmia before study drug administration) and one patient withdrew her consent. Thus, altogether 24 patients were exposed to the study drug and they all completed the 7-day infusion and the 2-week follow-up.

5.2 Baseline characteristics

Table 8 describes the demographics and main baseline characteristics of the patients in each study.

Table 8. Demographics and baseline characteristics of the study populations.

	Study I	**Study II**	**Study III A and B**	
Levosimendan treatment	**24-48 hours**	**0.2 μg/kg/min for 24 hours**	**0.05 μg/kg/min for 7 days**	**0.1 μg/kg/min for 7 days**
N	**98**	**12**	**12**	**12**
Age (years), mean±SEM	59 ± 1	62 ± 3	65 ± 3	60 ± 4
Sex, N (%)				
Male	79 (81)	10 (83)	10 (83)	12 (100)
Female	19 (19)	2 (17)	2 (17)	0 (0)
Weight (kg)	82 ± 2	78 ± 5	83 ± 5	80 ± 4
Height (cm)	175 ± 1	173 ± 3	174 ± 2	174 ± 3
Race, N (%)				
Asian	1 (1)			
African American	33 (34)			
Caucasian	60 (61)	12 (100)	12 (100)	12 (100)
Hispanic	3 (3)			
Other	1 (1)			
Etiology of heart failure, N (%)				
Ischemic	61 (62)	6 (50)	7 (58)	7 (58)
Non-ischemic	37 (38)	6 (50)	5 (42)	5 (42)
NYHA Class, N (%)				
III	65 (66)	11 (92)	11 (92)	9 (75)
IV	33 (34)	1 (8)	1 (8)	3 (25)
HR, bpm	80 ± 2	69 ± 6	68 ± 4	73 ± 6
SBP, mmHg	112 ± 2	125 ± 5	121 ± 5	115 ± 5
DBP, mmHg	70 ± 1	74 ± 3	73 ± 3	74 ± 3
LVEF, (%)	21 ± 1	30 ± 2	28 ± 2	28 ± 2
Use of selected drugs, N (%)				
Diuretics	97 (99)	11 (92)	11 (92)	12 (100)
ACE inhibitors/AT_1 blockers	92 (94)	11 (92)	12 (100)	12 (100)
Digitalis glycosides	86 (88)	12 (100)	11 (92)	10 (83)
Beta-blocking agents	4 (4)	8 (67)	8 (67)	7 (58)

For abbreviations, see page 7

Mean age of the patients was similar in all studies, approximately 60 years. Most patients were male, only 17% were female. Mean weight and height were similar

across the studies. All patients in the two European studies run in Finland and Estonia were Caucasian, and in the US study almost two thirds were Caucasian and one third African American.
More than half of the patients had coronary artery disease as an underlying cause for heart failure. In the US study, more patients were in NYHA class IV, their baseline heart rate was higher and blood pressure somewhat lower. Their left ventricular ejection fraction was also considerably lower. The patients in the US study had also a Swan-Ganz catheter placed for the measurement of right-side pressures and cardiac output. Their mean pulmonary capillary wedge pressure was 26.8±0.9 mmHg and cardiac output 3.6±0.1 l/min.
In all studies the study drugs were administered on top of the patients´ current medications. Almost all patients were treated with diuretics, ACE inhibitors or AT_1 blockers and with digitalis glycosides. Beta-blocking agents were used far more frequently in the European studies than in the US study. The frequency of patients with a beta-blocking agent as a concomitant medication varied from 58 to 67% in the European studies but was only 4% in the US study. There was also a considerable difference in beta-blocker use between patients in Finland and Estonia. Of the 19 Finns, 16 (84%) had a beta-blocking agent, whereas of the 17 Estonians, only 7 (41%) had a beta-blocking agent.

5.3 Hemodynamics

Study I

During phase 1 of the study (from 0 to 6 hours), levosimendan significantly improved the hemodynamic state of the patients when compared to placebo as reported by Slawsky et al (154) and shown in Table 9 and Figure 16. Pulmonary capillary wedge pressure, pulmonary and right atrial pressure, pulmonary and systemic vascular resistance significantly decreased and stroke volume and cardiac output significantly increased while there was no change in the placebo group. Heart rate also increased and systolic and diastolic blood pressure decreased in the levosimendan group.

The hemodynamics in the levosimendan group further improved during the open-label infusion in phase 2 of the study (from 6 hours to 24 hours), although the dose was halved. Pulmonary capillary wedge pressure, mean pulmonary artery pressure and systemic vascular resistance significantly decreased. Systolic and diastolic blood pressure also slightly but significantly decreased. In other hemodynamic indices the changes were not statistically significant. (Table 9).

The mean values of the hemodynamic indices at the beginning of phase 3 (at 24 hours) were similar in the levosimendan continuation and levosimendan withdrawal groups. Also at 48 hours the hemodynamics were similar in the continuation and withdrawal groups, and generally unchanged from hemodynamics at 24 hours (Table 10) with the exception of heart rate, which was higher ($p<0.001$) in both groups at 48 hours (versus 24 hours), and systemic vascular resistance, which was lower ($p=0.071$) in both groups at 48 hours (versus 24 hours).

Table 9. Baseline values and changes in the hemodynamic parameters at 6 hours and at 24 hours compared with baseline in levosimendan (n=98) and placebo (n=48) treated patients. Mean ± SEM are given.

Variable		**Baseline**	**△ 6 h***	**△ 24 h**	**p-value****
SV (ml)	LS all	46.3 ± 1.7	+12.9 ± 1.2	+16.1 ± 1.6	0.208
	Placebo	45.3 ± 2.4	-1.4 ± 1.8		
PCWP (mmHg)	LS all	26.8 ± 0.9	-6.4 ± 0.7	-8.9 ± 0.7	<0.001
	Placebo	27.5 ± 1.2	+0.1 ± 1.0		
CO (l/min)	LS all	3.6 ± 0.1	+1.4 ± 0.1	+1.6 ± 0.1	0.187
	Placebo	3.8 ± 0.2	-0.1 ± 0.1		
PAP (mmHg)	LS all	38.4 ± 1.1	-5.8 ± 0.7	-8.8 ± 0.8	0.002
	Placebo	39.2 ± 1.6	+1.0 ± 1.0		
RAP (mmHg)	LS all	10.8 ± 0.6	-3.1 ± 0.4	-3.7 ± 0.4	0.111
	Placebo	13.1 ± 0.9	+1.3 ± 0.6		
SVR (dyne-sec-cm^{-5})	LS all	1753 ± 65	-514 ± 50	-605 ± 50	0.033
	Placebo	1621 ± 92	+41 ± 72		
PVR (dyne-sec-cm^{-5})	LS all	290 ± 21	-80 ± 13	-95 ± 16	0.205
	Placebo	264 ± 30	+33 ± 19		
HR (bpm)	LS all	80 ± 1.7	+5.9 ± 0.8	+5.8 ± 1.0	0.219
	Placebo	84 ± 2.5	+0.8 ± 1.2		
SBP (mmHg)	LS all	112 ± 2.1	-1.4 ± 1.0	-6.6 ± 1.3	<0.001
	Placebo	110 ± 3.0	+2.4 ± 1.5		
DBP (mmHg)	LS all	70 ± 1.4	-5.5 ± 1.0	-9.1 ± 1.1	0.004
	Placebo	71 ± 2.0	+0.7 ± 1.5		

*) all changes were statistically significantly different between placebo and levosimendan at 6 hours when analyzed by ANOVA model with effects for treatment interaction ($p<0.001$ for all but SBP, for SBP $p=0.037$)

**) comparison between the values at 6 and 24 hours in levosimendan group when analyzed with paired t-test

LS = levosimendan

For abbreviations, see page 7

Table 10. Baseline values at 24 hours and change in hemodynamic variables from 24 h to 48 h in levosimendan continuation (n=42) and withdrawal groups (n=43). Mean ± SEM are given.

Variable		**24 h**	**p-value***	**△ 48 h**	**p-value***
SV (ml)	LS continuation	62.2 ± 3.3	0.885	+1.2 ± 2.2	0.558
	LS withdrawal	62.9 ± 3.2		-0.4 ± 2.2	
PCWP (mmHg)	LS continuation	18.0 ± 1.2	0.398	-0.3 ± 0.8	0.814
	LS withdrawal	19.5 ± 1.2		0.0 ± 0.8	
CO (l/min)	LS continuation	5.2 ± 0.2	0.990	+0.5 ± 0.2	0.333
	LS withdrawal	5.2 ± 0.2		+0.2 ± 0.2	
PAP (mmHg)	LS continuation	31.0 ± 1.4	0.754	-0.0 ± 1.0	0.790
	LS withdrawal	30.3 ± 1.4		+0.3 ± 1.0	
RAP (mmHg)	LS continuation	6.7 ± 0.7	0.166	-0.3 ± 0.5	0.649
	LS withdrawal	8.1 ± 0.7		+0.1 ± 0.6	
SVR (dyne-sec-cm^{-5})	LS continuation	1127 ± 64	0.670	-88 ± 46	0.956
	LS withdrawal	1166 ± 63		-84 ± 46	
PVR (dyne-sec-cm^{-5})	LS continuation	229 ± 18	0.100	-5 ± 15	0.986
	LS withdrawal	187 ± 18		-5 ± 15	
HR (bpm)	LS continuation	85 ± 2.4	0.945	+6.7 ± 1.6	0.145
	LS withdrawal	85 ± 2.5		+3.2 ± 1.7	
SBP (mmHg)	LS continuation	104 ± 3.1	0.302	+2.3 ± 1.9	0.425
	LS withdrawal	108 ± 3.2		0.0 ± 2.1	
DBP (mmHg)	LS continuation	61 ± 1.8	0.531	+1.1 ± 1.7	0.164
	LS withdrawal	62 ± 1.9		-2.5 ± 1.9	

*) p-values based on ANOVA model with effects for treatment interaction
LS = levosimendan
For abbreviations, see page 7

The changes in hemodynamics during the first 6 hours followed the increase in levosimendan concentrations. Thereafter, the changes in hemodynamics seem to follow also the increasing metabolite concentrations (Figure 16). Although the concentration of levosimendan decreases from 6 to 24 hours, stroke volume slightly but not significantly further increased and pulmonary capillary wedge pressure significantly further decreased ($p<0.001$). At the same time, the metabolite concentrations of OR-1896 and OR-1855 (not shown in Figure 16) start to build up in plasma. From 24 to 48 hours, the metabolites seem to account for the maintenance of the hemodynamic effects. However, a small but not significant attenuation in the hemodynamic effects from 24 to 30 hours is evident in the levosimendan withdrawal group indicating that the disappearance of levosimendan from the blood at least temporarily diminishes the hemodynamic effects.

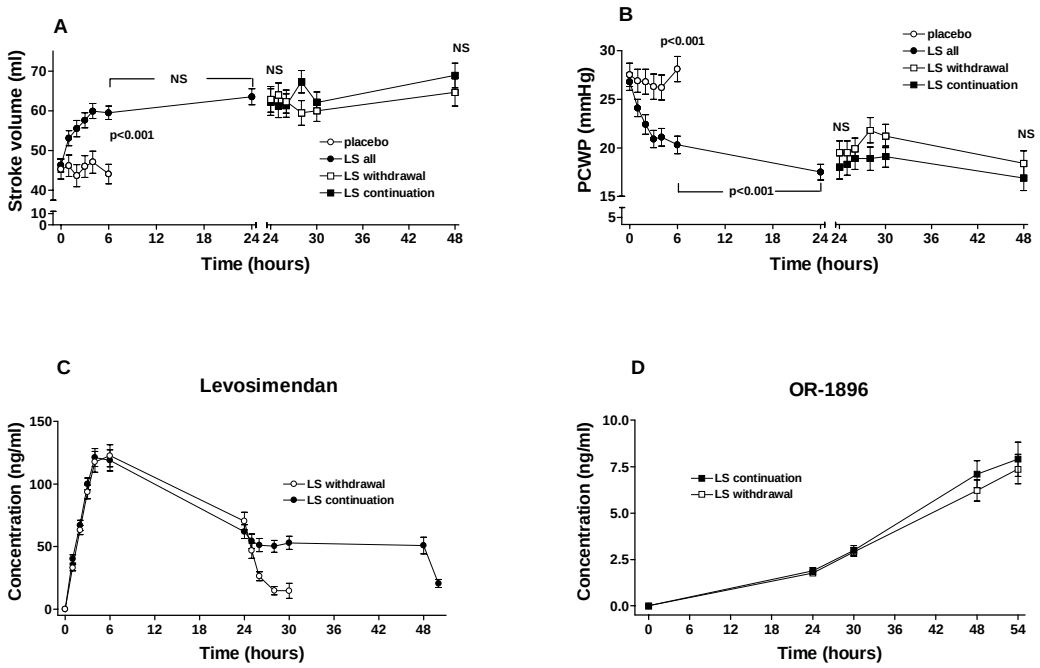

Figure 16. Mean (±SEM) changes in stroke volume (A), PCWP (B) and plasma concentration-time curves of levosimendan (C) and the metabolite OR-1896 (D) in study I.

Study II

The mean (±SEM) maximum heart rate increase determined from the 24-hour Holter recordings was 10±8 bpm ($p<0.005$). The mean heart rate remained statistically significantly elevated during the initial hospitalisation up to three days after stopping the study drug infusion. One week after stopping the infusion, the mean heart rate was no longer statistically significantly above the baseline (Figure 17).

When the Holter recordings were divided into 12-hour periods, it was seen that during the first 12 hours, the mean increase in heart rate was 2±10 bpm. During this time-period, the plasma concentrations of levosimendan had reached steady-state and only in two patients were very low concentrations of the metabolites observed. The maximum increase of 13±8 bpm in mean heart rate in this analysis was observed in the second 12-hour period, 12 to 24 hours after stopping levosimendan infusion. In this 12-hour period, levosimendan itself had disappeared from plasma, while the metabolite concentrations where almost at their maximum (Figure 17).

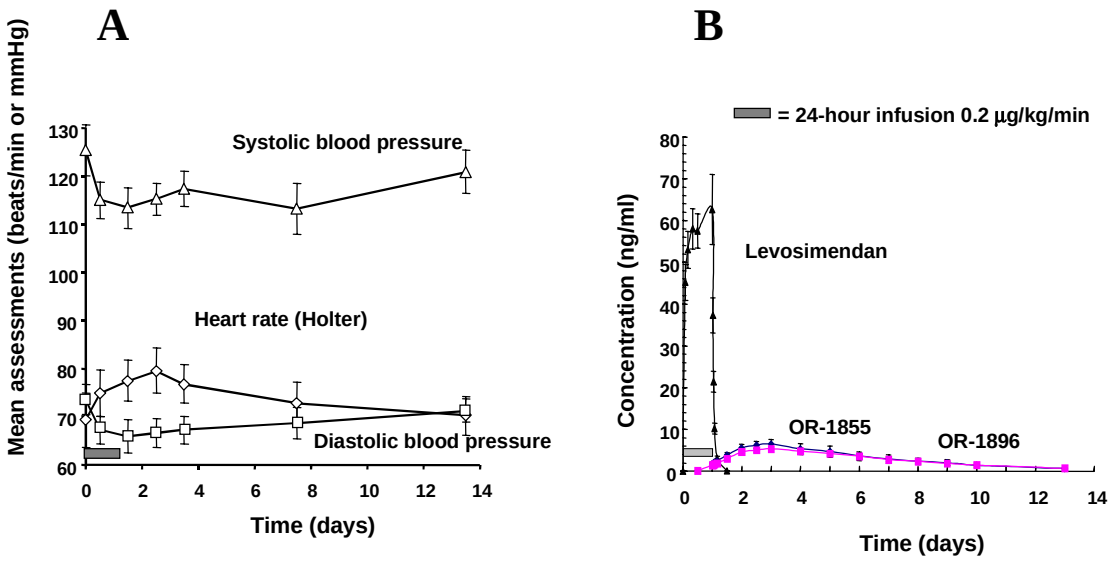

Figure 17. Mean (±SEM) values of the hemodynamic variables (A) and the plasma concentration-time curves of levosimendan, and the metabolites OR-1855 and OR-1896 (B) in study II.

The mean maximum decreases in systolic and diastolic blood pressure of 12±14 mmHg and 8±10 mmHg, respectively ($p<0.05$ for both), were observed during the first day after stopping the levosimendan infusion. The mean systolic blood pressure remained statistically significantly below the baseline during the initial hospitalisation up to three days after stopping the study drug infusion. The mean diastolic blood pressure was no longer significantly below the baseline on the third day after stopping the infusion.

Study III A

The mean (±SD) heart rate increased slowly during the infusion period (Figure 18). The maximum increases of 18±18 and 26±19 bpm, respectively, were observed at the end of the infusion period ($p<0.001$ for both groups). During the follow-up, the mean heart rate values decreased gradually, but still at the end of the 2-week follow-up period, the value was 9±11 bpm and 11±11 bpm above the baseline in the lower and higher levosimendan dose groups, respectively ($p<0.05$ for both groups). There was no statistically significant dose-response in heart rate ($p=0.07$), possibly due to the limited sample size, though the heart rate curves (Figure 18) suggest some trend.

The changes in heart rate closely followed the changes in the metabolite concentrations (Figure 18). The impact of levosimendan itself on heart rate at the plasma levels achieved in this study was minor. At 24 hours, the mean increase in heart rate was 2±12 and 6±10 bpm in the lower and higher levosimendan dose groups, respectively. At this time-point, the plasma levosimendan concentrations were at steady-state, and those of the metabolites OR-1896 and OR-1855 were very low or under the limit of determination in individual patients.

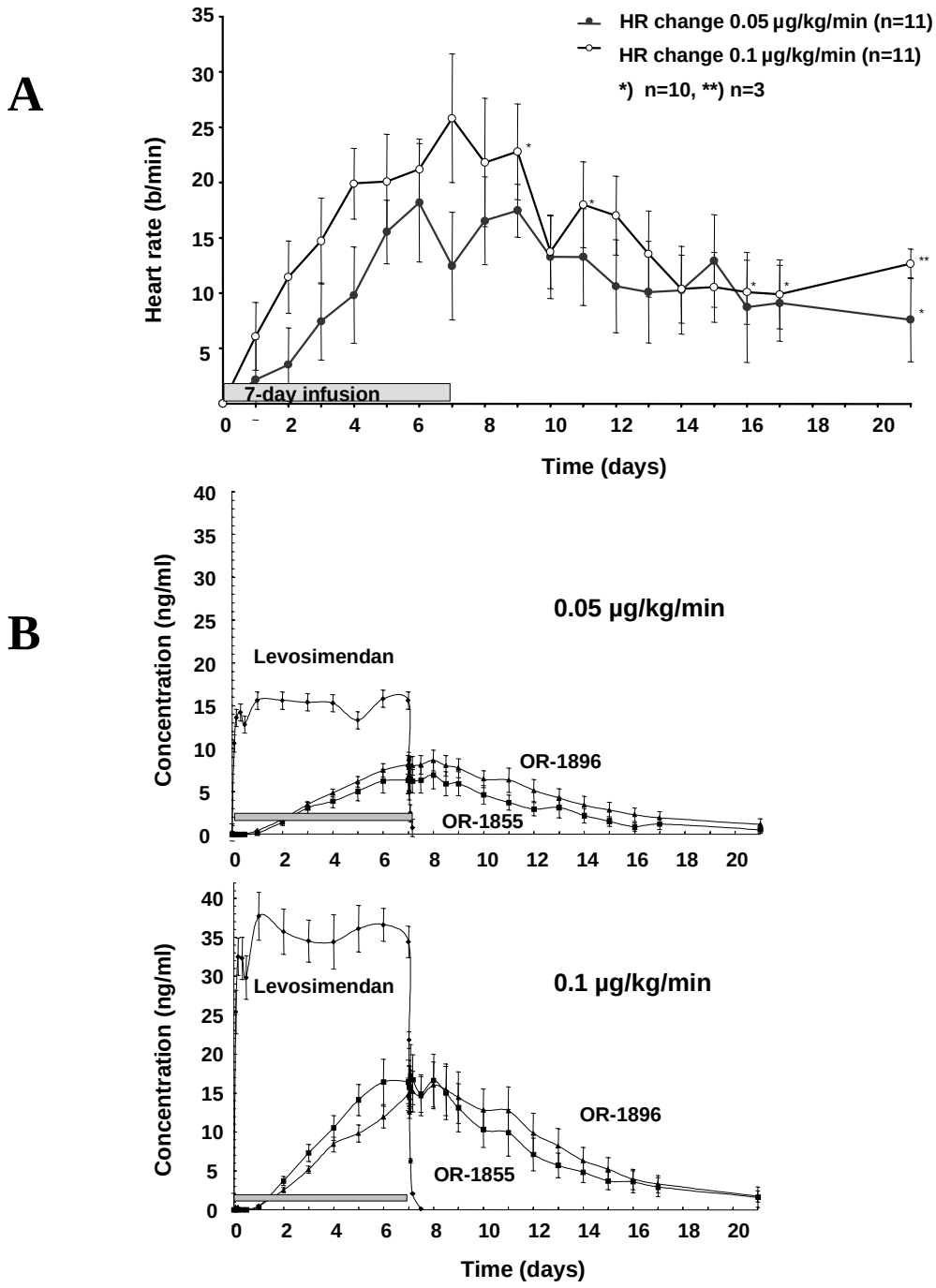

Figure 18. Changes in mean (±SD) heart rate values (A) and the plasma concentration-time curves of levosimendan, and the metabolites OR-1855 and OR-1896 (B) in study III A.

Additionally, correlations between heart rate and plasma concentrations of levosimendan and the metabolites OR-1855 and OR-1896 were calculated. The plasma concentrations of levosimendan achieved with 0.05 µg/kg/min or 0.1 µg/kg/min did not correlate with changes in heart rate (Figure 19). However, both OR-1896 (Figure 20) and OR-1855 (Figure 21) correlated significantly with heart rate changes.

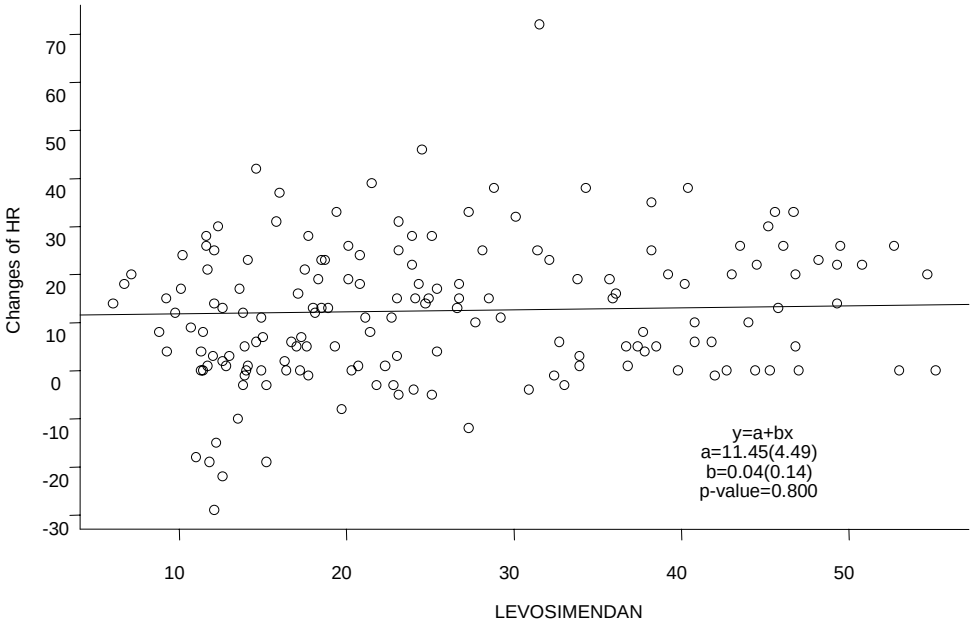

Figure 19. Correlation between levosimendan concentration (ng/ml) and changes in heart rate (bpm) during a 7-day infusion of levosimendan at infusion rates 0.05 µg/kg/min and 0.1 µg/kg/min (study III A).

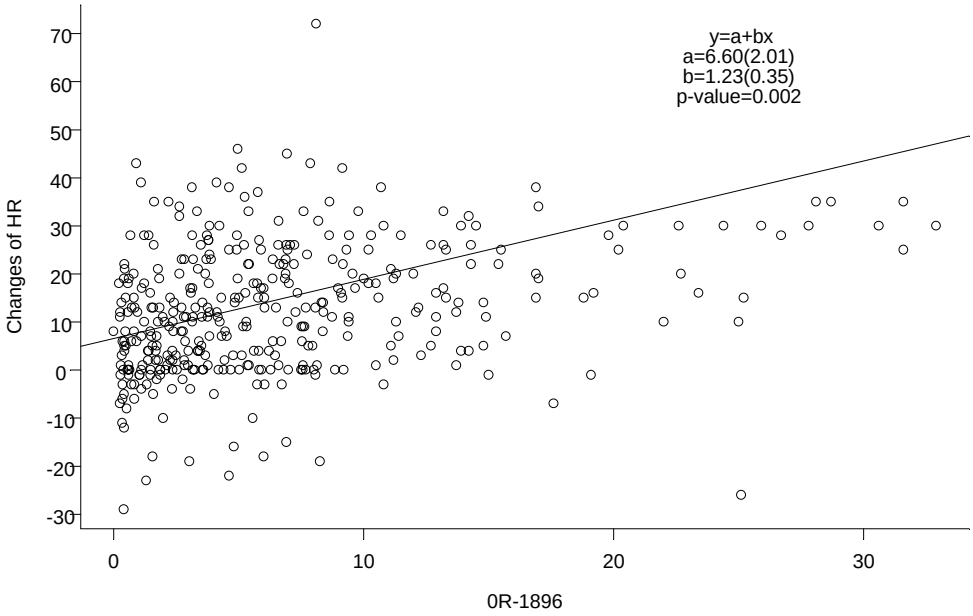

Figure 20. Correlation between OR-1896 concentration (ng/ml) and changes in heart rate (bpm) during a 7-day infusion of levosimendan at infusion rates 0.05 µg/kg/min and 0.1 µg/kg/min and the 10-15 days' follow-up (study III A)

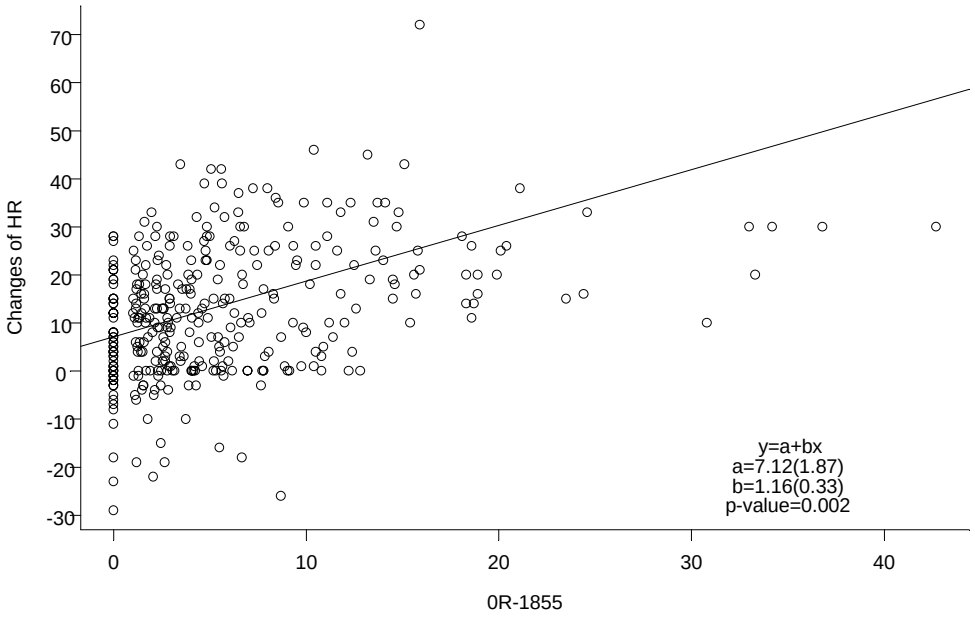

Figure 21. Correlation between OR-1855 concentration (ng/ml) and changes in heart rate (bpm) during a 7-day infusion of levosimendan at infusion rates 0.05 μg/kg/min and 0.1 μg/kg/min and the 10-15 days' follow-up (study III A).

The changes in blood pressure were relatively modest in the study. Mean systolic and diastolic blood pressure decreased maximally by 6±19 mmHg and 6±9 mmHg, respectively, in the lower levosimendan dose group ($p<0.05$). In the higher levosimendan dose group, the maximum decreases in systolic and diastolic blood pressure were 11±14 mmHg and 11±10 mmHg, respectively ($p<0.05$). The mean systolic blood pressure returned to baseline already during the infusion period in the lower levosimendan dose group. In the higher levosimendan dose group, baseline was reached within two days after stopping the infusion. The mean diastolic blood pressure returned to baseline within three days after stopping the infusion in the lower levosimendan dose group and by the end of the follow-up in the higher levosimendan dose group.

5.4 Symptoms

During the first 6 hours in study I, the patients treated with levosimendan reported significantly more often an improvement in dyspnoea and fatigue than those treated with placebo as reported by Slawsky et al (154) and shown in Table 11. The achieved improvement in symptoms of heart failure at 6 hours in the levosimendan group was followed by further increase in the percentage of patients reporting improved status during the open-label levosimendan infusion up to 24 hours. During the period from 24 hours up to 48 hours, the percentage of patients improving still increased but there were no significant differences in symptom evaluations between levosimendan continuation and withdrawal groups at 48 hours (Table 11). The physician´s rating was generally in line with that of the patient.

Table 11. Change in patients` ratings of symptoms of heart failure in study I.

Dyspnea		**Improved**		**Worsened**		
6 h*	Placebo (46)	15.2 %		17.4 %		
	LS all (89)	29.2 %		9.0 %		p=0.037[a]
24 h*	LS all (86)	37.2 %		11.6 %		
48 h**	LS continuation (41)	LS withdrawal (41)		LS continuation (41)	LS withdrawal (41)	p=0.924[b]
	9.8 %	14.6 %		9.8 %	9.8 %	
Fatigue		**Improved**		**Worsened**		
6 h*	Placebo (45)	22.2 %		11.1 %		
	LS all (89)	41.6 %		10.1 %		p=0.057[a]
24 h*	LS all (86)	39.5 %		10.6 %		
48 h**	LS continuation (41)	LS withdrawal (41)		LS continuation (41)	LS withdrawal (41)	p=0.238[b]
	22.5 %	19.5 %		22.5 %	9.8 %	

*) compared with baseline

**) compared with 24 hours

a) difference between levosimendan and placebo at 6 h

b) difference between continuation and withdrawal groups at 48 h

5.5 Pharmacokinetics

Pharmacokinetic variables

Pharmacokinetics were calculated in studies II and III B. The results are summarized in Tables 12-14.

The mean elimination half-life value for levosimendan was approximately one hour and for the metabolites OR-1855 and OR-1896 approximately 80 hours. The mean total clearance of levosimendan and the volume of distribution for levosimendan were similar after different doses. The mean steady-state concentrations (concentration at 24 h in study II) of levosimendan followed the increasing infusion rates. The mean AUC values of levosimendan and the metabolites increased dose proportionally.

Table 12. Mean (±SD) values for pharmacokinetic variables of levosimendan in studies II and III B.

	Study II	**Study III B**	
LS infusion rate	**0.2 µg/kg/min/24h (n=12)**	**0.05 µg/kg/min/7days (n=12)**	**0.1 µg/kg/min/7days (n=12)**
LS cumulative dose (mg)	**22.6 ± 6.1**	**41.8 ± 8.0**	**81.2 ± 13.9**
$t_{1/2el}$ (h)	1.26 ± 0.40	1.07 ± 0.15	1.38 ± 0.73
Cl_{tot} (l/h/kg)	0.22 ± 0.06	0.21 ± 0.05	0.18 ± 0.05
V_d (l/kg)	0.39 ± 0.11	0.33 ± 0.12	0.35 ± 0.18
C_{ss} (ng/ml)	62.6 ± 29.2*	14.9 ± 3.63	34.6 ± 8.50
$AUC^{0-\infty, trap}$ (ng· h/ml)	1392 ± 444	2507 ± 620	5966 ± 1482

$t_{1/2el}$ = terminal elimination half-life
CL_{tot} = total clearance
V_d = volume of distribution based on AUC
C_{ss} = concentration at steady state
AUC = area under the curve
*) concentration at the end of the infusion (24 h)

Table 13. Mean (±SD) values for pharmacokinetic variables of OR-1855 in studies II and III B.

	Study II	**Study III B**	
LS infusion rate	**0.2 µg/kg/min/24h (n=12)**	**0.05 µg/kg/min/7days (n=12)**	**0.1 µg/kg/min/7days (n=12)**
LS cumulative dose (mg)	**22.6 ± 6.1**	**41.8 ± 8.0**	**81.2 ± 13.9**
$t_{1/2el}$ (h)	74.8 ± 24.1*	72.6 ± 17.8	78.4 ± 27.8
C_{max} (ng/ml)	6.80 ± 4.1	7.76 ± 5.11	18.1 ± 11.2
$AUC^{0-\infty, trap}$ (ng· h/ml)	1263 ± 767*	1652 ± 1262	3947 ± 2418

$t_{1/2el}$ = terminal elimination half-life
C_{max} = maximum concentration
AUC = area under the curve
*) n=9

Table 14. Mean (± SD) values for pharmacokinetic variables of OR-1896 in studies II and III B.

	Study II	**Study III B**	
LS infusion rate	**0.2 µg/kg/min/24h (n=12)**	**0.05 µg/kg/min/7days (n=12)**	**0.1 µg/kg/min/7days (n=12)**
LS cumulative dose (mg)	**22.6 ± 6.1**	**41.8 ± 8.0**	**81.2 ± 13.9**
$t_{1/2el}$ (h)	77.4 ± 30.6	81.3 ± 37.1	81.2 ± 27.5
C_{max} (ng/ml)	5.51 ± 2.16	9.9 ± 4.7	17.1 ± 9.8
$AUC^{0-\infty,trap}$ (ng· h/ml)	948 ± 535	2371 ± 1825	3887 ± 2540

$t_{1/2el}$ = terminal elimination half-life
C_{max} = maximum concentration
AUC = area under the curve

Protein binding

The mean plasma protein binding value for levosimendan was 97% (range 95 to 98%), for OR-1855 39% (range 33 to 48%) and for OR-1896 42% (range 33 to 49%).

Excretion

Only negligible amounts of unchanged levosimendan were found in urine. The amount of OR-1855 excreted in urine 6-24 hours after stopping the 0.05 μg/kg/min levosimendan infusion was 21.0±16.4 μg (mean±SD). The corresponding amount for OR-1896 was 42.7±24.3 μg. This represents less than 1% of the dose administered for the previous 24 hours.

5.6 Safety assessments

Adverse events

Study I

During the placebo-controlled phase 1 of the study, the overall proportion of patients reporting an adverse event was similar in the placebo and levosimendan groups, 19% and 17%, respectively (154). During the open-label phase 2, both injection site pain and headache occurred in 7.1% of the patients and nausea in 6.4%. Ventricular tachycardia occurred in 2.1% of the patients. During phase 3, the levosimendan continuation group had more frequent reports of headache (21.0% vs 14.0%), nausea (7.1% vs 2.3%), and vomiting (4.8% vs 0.0%) than the withdrawal group. Other adverse events were reported with similar frequency in the levosimendan continuation and withdrawal groups. Hypotension occurred more frequently in the withdrawal group (7.0% vs 4.8%). One case of ventricular tachycardia occurred in the withdrawal group but none were reported in the continuation group.

No deaths occurred during the 48-hour infusion period. During the 14-day follow-up, 5 deaths occurred: 3 in patients randomized to placebo for the initial 6-hour infusion period (6.3%) and 2 in patients randomized to levosimendan (2.0%).

Study II

Six adverse events in four of the 12 patients were reported. They were considered mild in five and moderate in one case. Two patients reported headache during the infusion. One patient experienced palpitations starting 14 hours after the initiation of levosimendan infusion and lasting for a total of 25 hours. In Holter recordings of this patient, no other relevant findings except a considerable increase in heart rate from 58 bpm to 78 bpm were observed. The same patient also had flu during the follow-up period, the symptoms of which resolved within two days. There was also one case of mild backache and one case of subjective swellings in fingers.

Study III (A and B)

In all patients the continuous 7-day infusion could be completed without interruptions other than for practical reasons (shower etc.). Adverse events were reported in 16 of

the 24 patients, in 8 patients in each group. One patient experienced worsening of heart failure (shortness of breath and nausea) 10 days after stopping the levosimendan infusion of 0.1 µg/kg/min. He was hospitalised for 4 weeks, during which time the event resolved. The event was considered not to be related to levosimendan by the investigator. All the other adverse events were graded mild to moderate. The most common adverse events were rhythm disorders and infusion site reactions, each occurring in 4 patients. The reported rhythm disorders were sinus tachycardia, ventricular extrasystoles and bigeminy. Hypotension, headache and dizziness occurred in 3 patients and angina pectoris, swellings in lower extremities and rash in 2 patients.

Safety laboratory variables

Study I

Levosimendan had little effect on most laboratory assessments performed in the study. Patients treated with levosimendan showed slight but statistically significant decrease in serum potassium when compared to values from the placebo group at 6 hours; -0.34 mmol/l versus -0.14 mmol/l ($p=0.041$). At 48 hours, there was no significant difference in the change in the levosimendan continuation and withdrawal groups. Blood hemoglobin decreased similarly in levosimendan and placebo groups at 6 hours, by 7.7 g/dl and 6.2 g/dl, respectively, with no significant difference between the groups. At 48 hours, the changes were similar in the levosimendan continuation and withdrawal groups.

Study II

Changes in the safety laboratory parameters during the study were minor. There was a slight decrease in red cell parameters. The mean blood hemoglobin decreased non-significantly by 5±8 g/l during the infusion. At the end of the 2-week follow-up, the mean blood hemoglobin was still slightly but not significantly below the baseline. No signs of hemolysis were observed in the laboratory parameters as evidenced by the practically unchanged values of serum haptoglobin, reticulocyte count, serum lactate dehydrogenase and serum bilirubin during the study. Serum potassium values were also virtually unchanged during the study.

Study III (A and B)

Changes in the safety laboratory parameters during the study were minor. There was a modest decrease in red blood cell parameters. The mean blood hemoglobin decreased by 8±12 g/l (from 142±11 g/l to 134±10 g/l) and by 9±8 g/l (from 149±22 g/l to 140±17 g/l) by the end of the infusion in the lower and higher levosimendan dose groups, respectively ($p<0.05$ for both groups). By the end of the follow-up, the mean blood hemoglobin was no longer statistically significantly below the baseline in either group. Changes similar to those in blood hemoglobin were observed in mean blood hematocrit and blood red cell count. No significant changes in serum potassium were observed.

Electrocardiograms

Study I

The changes in PR, QRS and QT intervals were minor and no significant changes were seen at 6 hours when levosimendan was compared to placebo and at 48 hours when levosimendan continuation and withdrawal groups were compared. Mean QTc (Bazett) increased from the baseline value of 447±5 ms to 464±4 ms at 6 hours in the levosimendan group and from 442±7 ms to 455±6 ms in the placebo group (p=0.275). At 24 hours, mean QTc in the levosimendan group was 461±6 ms. At 48 hours, the values were 459±6 and 458±6 in the levosimendan continuation and withdrawal groups, respectively (p=0.904).

Study II

There were no statistically significant changes in PQ, QRS, QT or QTc intervals during the study. The mean QTc interval by Bazett´s equation increased slightly but not significantly by 27±25 ms during the study. The maximum increase was observed at the end of the infusion. Thereafter, the mean QTc interval slowly declined to baseline level during the follow-up.

Study III (A and B)

The mean PQ interval decreased by 10±15 ms and 20±27 ms in the lower and higher levosimendan dose groups, respectively ($p<0.05$ for both groups). There were no statistically significant changes in the mean QRS intervals during the study. The mean QT interval decreased slightly but not significantly by 10±40 ms and 16±22 ms in patients receiving 0.05 µg/kg/min and 0.1 µg/kg/min, respectively. The mean QTc values by Bazett´s equation increased by 38±42 ms and 52±40 ms in the lower and higher levosimendan dose groups, respectively ($p<0.001$ for both groups). The decrease of the QT interval and the increase of the QTc interval closely followed the increase of heart rate.

Holter monitoring

Holter monitoring was performed only in study II. No sustained VTs or ventricular fibrillations were observed in any of the Holter recordings. There was a considerable interindividual variation in the number of VT runs in the Holter recordings. The median number of VT runs per day was 3.0 at baseline. During the infusion and the following 3 days, the medians were 2.5, 4.0, 5.0, and 4.5, respectively, and at the end of the follow-up the median was 2.5. The change in the median number of VT runs during the whole follow-up period was not significant (p-value for the squared time effect 0.52).

6. DISCUSSION

6.1 Study subjects

The study for publication I was performed in the USA and this is the only study in which patients other than Caucasian were recruited. The other studies were run in Finland and Estonia and all patients were Caucasian. The nature of the US study also differed from the other studies. It was an efficacy study whereas the other studies focused more on pharmacokinetics and basic hemodynamics. Consequently, the patients in the US study had more severe heart failure with more patients in NYHA class IV and lower baseline ejection fraction.

The baseline treatment of the patients largely followed the recommendations of the European and US guidelines (19, 20). Almost all patients were treated with ACE inhibitors or AT_1 blockers. The severity of the disease was reflected by the frequent use of diuretics (92-100%) and digitalis (83-100%) in different studies. The baseline medication included beta-blockers much more frequently in the European studies than in the US study. A plausible explanation for this difference is the time when the studies were performed. The recruitment of the patients in the US study took place from 1995 to 1997 and the European studies from 1998 to 1999. The first large-scale trial showing the beneficial effect of beta-blockers on mortality in chronic heart failure was published only in 1996 (4). There are also geographical differences in the usage of certain medications for heart failure (171) and traditionally beta-blockers have been used much more frequently in Scandinavia than in the rest of Europe and especially the USA. There was also a difference between the Finnish and Estonian patients in that more Finnish patients received beta-blockers, which is probably also explained by differences in therapeutic praxis, although the numbers were too small to draw any firm conclusions.

Male patients clearly outnumbered female patients in the studies. This is a well-recognised feature in clinical trials in heart failure patients - there seems to be a selection of younger male patients in these studies. The characteristics of the "average" heart failure population are considerably different. This is at least partly explained by the fact that studies usually include patients with systolic heart failure, who tend to be younger males. Diastolic heart failure patients in contrast are typically older females. Additionally, studies are performed more often in university clinics in younger patients. Investigators may also intentionally or unintentionally select younger and thus male patients to the studies (172-174).

6.2 Hemodynamic effects

The hemodynamic effects of levosimendan on invasively measured variables were studied only in study I. The changes in cardiac output, PCWP and systemic vascular resistance in levosimendan-treated patients were similar to those obtained in an earlier dose-finding study (153) and in an active comparator study (36). For example, PCWP was approximately 30% below baseline at 24 hours in all studies with approximately the same levosimendan doses.

Mean heart rate increased similarly in study I (6 bpm) as in the active comparator study (6 bpm) at 24 hours (36). In the earlier dose-finding study, the increase in heart rate at 24 hours with the dose of 0.2 μg/kg/min was, however, much higher, 13 bpm (153). This, however, is in line with the change seen in study II (13 bpm as evaluated by 12-hour Holter analysis). A plausible explanation for this difference is that the patients in study I and in the active comparator study had highly elevated preload at baseline as shown by mean PCWP values of, respectively, 26.8 mmHg and 24.8 mmHg in levosimendan-treated patients. On the other hand, the mean baseline PCWP was only approximately 15 mmHg in the dose-finding study. Although not measured in study II, it is likely that PCWP would have been at about the same level in this study as in both studies almost all patients had stable NYHA class III symptoms and the mean ejection fraction was similar. It is therefore likely that in study II and in the earlier dose-finding study, the considerable increase in heart rate was a consequence of preload dropping too low causing reflectory tachycardia. The same applies also to study III with prolonged infusions. As in earlier studies, systolic and diastolic blood pressure decreased only modestly in the present studies (36, 153).

The duration of the hemodynamic effects after levosimendan infusion has not been studied earlier. The results of study I show that the beneficial hemodynamic effects on invasively measured hemodynamic variables including PCWP, stroke volume and cardiac output last at least 24 hours after stopping a 24-hour levosimendan infusion. It is, however, likely that the beneficial effects last for some additional days thereafter. This is supported by the fact that there were no signs of fading in any of the measured hemodynamic variables at 48 hours and that in study II, the effect on heart rate was seen for several days after the 24-hour infusion.

The results of the thesis suggest that the hemodynamic effects observed at the beginning of the levosimendan infusion derive from the parent drug itself. Measurable amounts of the metabolites are seen in plasma at the earliest 12 hours after starting the infusion. Thereafter, with continued levosimendan infusion, the hemodynamic effects are most likely a sum of the effects of the parent drug and the metabolites. In study I, the hemodynamic effects were more pronounced at 24 hours than at 6 hours, although the dose of levosimendan had been halved at 6 hours and the plasma level of levosimendan had decreased accordingly.

It seems that the heart rate increasing effect of levosimendan is more modest than that of the active metabolite at the infusion rates used in the present studies. In study III A, the mean heart rate increase at 24 hours when the metabolite plasma levels were low was 2 bpm and 6 bpm in the lower and higher levosimendan dose groups, respectively, whereas the maximum heart rate increases of 18 and 26 bpm, respectively, coincided with the maximum metabolite levels. The plasma metabolite concentrations, but not the concentrations of the parent drug, were shown to correlate with heart rate values. In study II, the mean heart rate increased by 2 bpm during the first 12 hours and only thereafter was the considerable heart rate increase seen. On the other hand, in study I with the highest levosimendan infusion rates and highest plasma levels of levosimendan in the beginning of the study, mean heart rate increased by 6 bpm already at 6 hours. Thus, also levosimendan increases heart rate and the effect seems to correlate with the dose. This has also been shown in earlier studies in which higher levosimendan doses were used (159, 175). Preclinical data has also shown that both levosimendan (142, 161) and the metabolite OR-1896 (148, 149, 162) increase heart rate. The exact mechanism is, however, not known.

Whether both metabolites are hemodynamically active in man cannot be answered by the results of the present studies. In preclinical studies OR-1896 has been shown to be active and to have hemodynamic effects similar to those of levosimendan (148, 149, 162) while OR-1855 seems to be much less active. Therefore, it is probable that rather OR-1896 than OR-1855 caused the prolonged hemodynamic effects seen in the present studies.

The presence of an active metabolite has important clinical consequences. Dobutamine and milrinone, the most widely used intravenous inotropic agents, do not have known active metabolites. The effects of these drugs disappear logically after stopping the infusion according to the elimination half-life of the parent drug. When levosimendan is dosed, the hemodynamic effects may be seen several days after stopping the infusion. However, most of the adverse events are seen during and not after stopping the infusion, as shown in studies II and III (A and B).

6.3 Symptoms

Hemodynamic improvement in study I was accompanied by improvement in symptoms of heart failure. This is in line with the results seen in the active comparator study, the LIDO study (36). The fact that the patients were invasively monitored may, however, diminish the credibility of the results. The knowledge of improvement in hemodynamic variables may affect the evaluation of symptoms. Especially the physician´s evaluation could be biased, but the information may also pass on to a patient. This possibility is highlighted by the results with nesiritide in the VMAC study. Symptomatic improvement was significant in those nesiritide-treated patients who were monitored invasively, whereas no significant difference between nesiritide and placebo was seen in patients who were not (98).

6.4 Pharmacokinetics

The pharmacokinetics of levosimendan was similar in the present studies to that seen after bolus doses in healthy volunteers and in patients with stable congestive heart failure (38-40). The elimination half-life of approximately one hour has been a consistent finding in both healthy volunteers and patients. Accordingly, steady-state plasma concentrations will be reached within 4 hours as was shown in study III B. There was also a dose-proportional increase in the plasma concentrations of levosimendan with the different infusion rates in studies II and III B, which has been seen also after different bolus doses (164).

Only sparse data on the pharmacokinetics of the metabolites OR-1855 and OR-1896 was available before the present studies. In preclinical studies, the elimination half-life of the metabolite OR-1896 was shown to be 4.7 to 6.5 hours and in clinical studies increasing plasma levels of the metabolites were observed after stopping a 24-hour levosimendan infusion. In the present studies II and III B, the elimination half-life of the metabolites was shown to be 70 to 80 hours. Therefore, steady-state concentrations of the metabolites should be achieved only after about 2 weeks' continuous infusion. Thus, it is most likely that steady state was not reached with the 7-day infusion. The long elimination half-life also explains the fact that still at the end of the 2-week follow-up, low concentrations of the metabolites were seen in studies II and III B.

Levosimendan was shown to be 97% bound to plasma proteins while the metabolites were only approximately 40% bound to plasma proteins. Therefore, the free fraction of OR-1896 and OR-1855 in blood is up to 30 times higher than that of the parent drug. OR-1896 and levosimendan possess similar positive inotropic effects at the same concentrations in guinea pig hearts (162). As a consequence, the lower total plasma concentrations of OR-1896 to those of levosimendan can be expected to achieve considerable hemodynamic effects as demonstrated in the present studies.

Although pharmacokinetics were not calculated in study I, the plasma concentration-time curves of levosimendan and the metabolites followed the same pattern as in studies II and III B. However, due to the limited number of time-points for blood sampling in the elimination phase of levosimendan after 6 hours, the plasma concentration-time curves are most likely somewhat misleading for levosimendan. It is likely that the concentrations of levosimendan decrease more rapidly after 6 hours after the dose was halved than shown in Figure 16. This is evident from the shape of the concentration curves after 24 hours and 48 hours.

6.5 Safety aspects

Levosimendan was well-tolerated in the studies. The recommended duration of infusion in clinical practice is 24 hours. However, even the 7-day continuous infusion in study III was tolerated reasonably well and no premature discontinuations of the study drug infusion occurred despite a considerable increase in heart rate.

In the present studies, the continuous infusion rates varied mainly between 0.05 μg/kg/min and 0.2 μg/kg/min, and only in study I in the majority of the patients was the dose uptitrated temporarily to 0.4 μg/kg/min. In earlier studies, the safety profile of levosimendan has been good up to continuous infusion rates of 0.2 μg/kg/min. Higher infusion rates than 0.2 μg/kg/min have been associated with higher incidence of adverse events, especially hypotension and tachycardia (37, 153). The most common adverse events related to levosimendan infusion, headache and hypotension, are likely consequences of vasodilatation. These were seen also in the present studies.

Levosimendan was not associated with increased frequency of potentially malignant rhythm disorders in study II with Holter monitoring. This is in agreement with previous observations. In a meta-analysis of Holter recordings of altogether 236 heart failure patients, there was no difference between levosimendan and placebo in the percentages of patients having proarrhythmic events (34).

Deaths were seen only in study I, three (6.3%) in patients randomized to placebo and two in patients randomized to levosimendan (2.0%). Although the number of the deaths is far too small to draw any conclusions from, the results are similar to those seen in other levosimendan studies. Levosimendan-treated patients had a significantly lower mortality rate at 31 days when compared to dobutamine-treated patients with decompensated chronic heart failure and at 14 days when compared to placebo-treated patients with left ventricular failure after acute myocardial infarction (36, 37).

The changes in conduction intervals were similar to those observed in earlier levosimendan studies (151). Heart rate correction of the QT interval (QTc) was performed using Bazett´s equation as this is the formula required by the European regulatory authorities. The considerable increase in the QTc interval in study III A reflects, most likely, the inadequacy of the Bazett´s equation in correcting the QT

interval properly when heart rate increases considerably (157, 158). The use of e.g. Fredericia´s equation would have resulted in more modest prolongation. An electrophysiology study in patients has shown that levosimendan did not influence the QT interval when heart rate was kept constant by atrial pacing (159).

The modest decreases in red blood cell parameters observed in studies II and III and in serum potassium in study I are in line with similar modest changes in earlier studies (36, 153). Studies II and III, with prolonged follow-up, showed that the decrease in red cell parameters was only temporary. The exact mechanism of this decrease in red cell parameters is not known. However, hemolysis as an underlying cause can be ruled out by the unchanged values of serum haptoglobin, reticulocyte count, serum lactate dehydrogenase and serum bilirubin in study II. The phenomenon can be a reflection of hemodilution.

6.6 Dosage of levosimendan in the studies

Levosimendan is approved for marketing in several European countries. The recommended dosing is a bolus dose of 12-24 μg/kg in 10 minutes followed by continuous infusion of 0.1 μg/kg/min up to 24 hours. The dose may be increased up to 0.2 μg/kg/min or down titrated to 0.05 μg/kg/min, if required.

In study II, the dose was practically the same as the maximum recommended dose. In this study, the mean maximum concentration of levosimendan was approximately 60 ng/ml and that of the metabolite OR-1896 5.5 ng/ml.

In study I, with forced up-titration up to 0.4 μg/kg/min in the initial phase, the mean levosimendan concentration was increased up to approximately 120 ng/ml. Due to the relatively short follow-up, the maximum concentration of OR-1896 was probably not seen, but at the final blood sampling time-point at 54 hours, the mean concentrations of OR-1896 were 7.4 ng/ml and 7.9 ng/ml in patients receiving levosimendan for 24 hours and 48 hours, respectively. For some patients, this dosing seemed to be too high, as evidenced by the relatively high number of patients in whom the drug had to be discontinued prematurely due to exaggerated hemodynamic response.

In study III with 7-day infusions, the levosimendan concentrations remained relatively low but the mean maximum concentrations of OR-1896 increased up to 9.9 ng/ml and 17.1 ng/ml with the lower and higher dose, respectively. In both groups the increase in heart rate was considerable probably suggesting too high metabolite concentrations.

The results of the studies indicate that exaggerated hemodynamic response with levosimendan may be a consequence not only of a higher than recommended infusion rate, but also of extended infusions leading to accumulation of the active metabolite. The free fraction of the metabolite in blood is up to 30 times higher than that of the parent drug and therefore even a modest increase in the plasma concentration of the active metabolite has considerable hemodynamic effects. Thus the recommended maximum duration of 24 hours for intravenous levosimendan seems rational also according to the results of the studies presented. However, with lower infusion rates, also longer infusion durations may be possible as long as the metabolite accumulation is not too high.

6.7 Limitations of the studies

The hemodynamic follow-up in study I was relatively short in light of the current knowledge on the prolonged effects of the metabolites. The risks with prolonged catheterisation (e.g. arrhythmias and septicaemia) were the main reason for the short follow-up, but also at the time there was no knowledge that the metabolites would have such a long elimination half-life. The presence of a placebo group throughout the follow-up would also have improved the reliability of the hemodynamic evaluation in this study. The patients were, however, severely ill and a 48-hour placebo infusion was considered unethical.

Studies II and III (A and B) were open-label studies with no control group. This does not compromise the pharmacokinetic value of the studies. However, the hemodynamic evaluation would have been more reliable with a double-blind, placebo-controlled design. It was, however, deemed ethically questionable to expose still another group of patients to a 7-day continuous placebo infusion in study III. On the other hand, in study II, the accuracy of the hemodynamic evaluation was improved by determining the heart rate from repetitive 24-hour Holter recordings and measuring blood pressure 4 times daily. One could also criticise that only non-invasive hemodynamics (heart rate and blood pressure) were followed in studies II and III. It is, however, impossible to monitor patients invasively for several days, not to mention 2 weeks. The above risks of catheterisation would have been extremely high during the prolonged follow-up and therefore the extended catheterisation was deemed ethically unacceptable.

Most of the patients in studies II and III were clinically stable and thus not in need of intravenous inotropic support. No efficacy variables were utilised in these studies. However, the results of the studies, combined with the invasive efficacy data from study I, give valuable information on the duration and magnitude of the hemodynamic effects one could expect in clinical practice.

7. SUMMARY AND CONCLUSIONS

1. Levosimendan treatment was associated with prolonged hemodynamic effects beyond the infusion period in patients with severe congestive heart failure. With a 24-hour levosimendan infusion, the hemodynamic effects were maintained for several days after stopping the infusion. The prolongation of the effects after the infusion period followed the formation and elimination of the levosimendan metabolites OR-1855 and OR-1896. The latter metabolite has been shown to have similar hemodynamic effects to those of levosimendan in preclinical studies, and on the basis of the present studies, it seems to be active also in man.

2. The observations on the pharmacokinetics of the parent drug levosimendan were in concordance with the earlier data gathered after mostly bolus doses. The elimination half-life of levosimendan was one hour and the plasma levels and AUC of the drug increased dose-proportionally with continuous infusions up to 7 days. The binding to plasma proteins was also the same as found earlier, approximately 97%.

3. The pharmacokinetics of the levosimendan metabolites OR-1855 and OR-1896 was characterised by the long elimination half-life of approximately 70-80 hours. The plasma levels of the metabolites increased slowly and the maximum concentrations were seen only after an average of 2 days following the stop of a 24-hour infusion of levosimendan. The metabolites were only about 40% bound to plasma proteins, making their free fraction in the blood considerably larger than that of the parent drug.

4. The recommended duration of levosimendan treatment is up to 24 hours with intravenous administration. Even the 7-day infusions were reasonably well tolerated in the present studies with no premature discontinuations of the infusions. The metabolite levels, however, increased considerably and were associated with a marked increase in heart rate, which could be risky in unstable patients. Also the 48-hour infusion seemed not to achieve any benefits over the 24-hour infusion and was associated with slightly more adverse events. Therefore, the results of the present thesis support the administration of levosimendan only up to 24 hours.

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Matti Kivikko

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