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PHARMACODYNAMICS

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Haemodynamic interactions of a new calcium sensitizing drug levosimendan and captopril

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Abstract. *Objective*: Levosimendan in a new inodilator drug that sensitises troponin C in heart muscle cells to calcium, thus improving contractility. In previous studies, a single 2 mg intravenous dose of levosimendan increased cardiac output (CO) in healthy volunteers by about 40% and decreased pulmonary capillary wedge pressure in heart failure patients by 40–50%.
The aim of the present, double-blind study was to evaluate the safety of concomitant use of levosimendan and an ACE-inhibiting drug.
Methods: The haemodynamic effects of levosimendan, given with or without captopril, were evaluated by using 2-dimensional echocardiography, repeated blood pressure measurements and by ambulatory ECG recordings. Twenty-four male patients with stable NYHA II-III heart failure (EF < 40%) after a previous myocardial infarct were given, in randomised order, a single IV infusion of levosimendan or placebo. The infusions were repeated after 2 weeks treatment with upto 50 mg b.i.d. of captopril. Twelve patients received levosimendan 1 mg and twelve received 2 mg.
Results: Mean CO was increased from 6.0 to 6.8 $l \cdot min^{-1}$ in patients receiving 1 mg levosimendan compared to placebo, but only from 6.3 to 6.5 $l \cdot min^{-1}$ in patients receiving 2 mg. The increase in CO was statistically significant when all levosimendan patients were compared to placebo. Heart rate did not change after either dose. Mean stroke volume increased significantly after 1 mg but not after 2 mg of levosimendan. The addition of captopril did not change the effects of levosimendan. No additional decrease in systolic or diastolic blood pressure was observed when levosimendan and captopril were given concomitantly.
Conclusion: It seems that concomitant treatment with captopril does not change the haemodynamic effects of levosimendan. No adverse haemodynamic interactions were seen.

Key words Levosimendan, Captopril; calcium sensitizer, inotropic drugs, haemodynamics, heart failure, drug interaction

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Introduction

Calcium sensitisers are a new group of inodilator drugs that improve myocardial contractility by potentiating the effects of calcium ions on myofilaments [1]. Their primary goal is to increase the force of contraction by increasing the number of active actin-myosin cross-bridges per time during systole without affecting the availability of calcium ions. Calcium sensitiser drugs can either change the calcium affinity of the myofilament calcium receptor, troponin C, stabilise the changes in troponin induced by calcium binding, or just change actin-myosin cross-bridge kinetics. Changes in calcium affinity will lead to an increase in troponin-bound calcium ions and subsequently to an increase in active cross-bridges per time by prolonging the attachment time of one cross-bridge, but on the other hand, the reuptake of calcium might be impaired and relaxation time prolonged [2]. Stabilisation of calcium-induced changes will also lead to an increase in cross-bridge numbers per time, but without a change in relaxation kinetics since the calcium affinity of troponin is not changed. Actin-myosin cross-bridge kinetics can be changed by activating myosin-ATPase, which will lead to increased cycling-rate and formation of cross-bridges per time, but the increased ATP hydrolysis will subsequently increase the energy demand. Drugs known to affect the calcium sensitivity of contractile proteins utilise one or more of these mechanisms [3]. In addition, drugs that have been tested

clinically (pimobendan, MCI-154 and, levosimendan) also have additional vasodilator action, which might partly be related to their phosphodiesterase inhibitory action [4–6].

Levosimendan is a pyridazinone dinitrile that stabilises the calcium induced conformational change in troponin C [7]. In healthy volunteers a single IV dose of 1–5 mg levosimendan dose-dependently increased ejection fraction and cardiac output [8]. In patients with left ventricular dysfunction, the increase in cardiac output was less prominent, but there was a marked reduction in preload even after single IV dose of 0.5 mg levosimendan [6]. The haemodynamic effects correlate well with the plasma drug level and last for 2–4 h after a single dose [9]. Plasma levels below 100 ng · ml^{-1} have little effect on heart rate, but at concentrations above that level there is a dose-dependent positive chronotropic effect, that is probably related to the PDE inhibitory action of the drug. Since levosimendan is highly protein bound, its haemodynamic effects in vivo are seen with a free fraction of the drug of 1–2 ng · ml^{-1} [10]. ACE-inhibiting drugs are one of the primary therapies given to heart failure patients. It is important to see whether ACE-inhibitors given concomitantly with inodilator drug may induce potentially harmful excessive vasodilatation. In previous dose-ranging studies [6, 10], levosimendan has been administered to some patients receiving ACE-inhibiting drugs without problems. The present study was planned primarily to evaluate the safety of the concomitant administration of therapeutic IV doses of levosimendan with an ACE-inhibitor.

Patients and methods

Study design

The study was a double-blind, placebo controlled, crossover Phase II study comprising two parallel patient groups receiving different doses of levosimendan (1 mg or 2 mg) or placebo (Fig. 1). The patients received first a single IV dose of 1 mg or 2 mg levosimendan and placebo (diluted in 5 % glucose and given as a 10 min infusion with an infusion pump), in random order, with an intervening wash-out period of 24 hours. After that, all the patients received captopril (Capoten, Bristol-Myers-Squibb) treatment for 2 weeks in order to reach complete ACE-inhibition. The dose of captopril was titrated up to 50 mg bid (6.25 mg–12.5 mg–25 mg–50 mg) at three day intervals according to the clinical response. If the diastolic blood pressure were < 70 mmHg or the systolic blood pressure < 100 mmHg, the dose of captopril was reduced to the previous level. The patients received the highest dose of captopril for at least 3 days in order to reach a haemodynamic steady state. They were then given levosimendan and placebo for the second time, again in random order, with a wash-out period of 24 h in between.

The study protocol followed the guidelines of the Declaration of Helsinki. After receiving both oral and written information about the study and the treatments, the patients were asked to sign a letter of consent. Only thereafter were they eligible for recruitment. Despite the consent, a patient had the right to withdraw whenever he so wished without stating a reason. The approval of the Ethics Committee of Tarto University was obtained before initiation of the study.

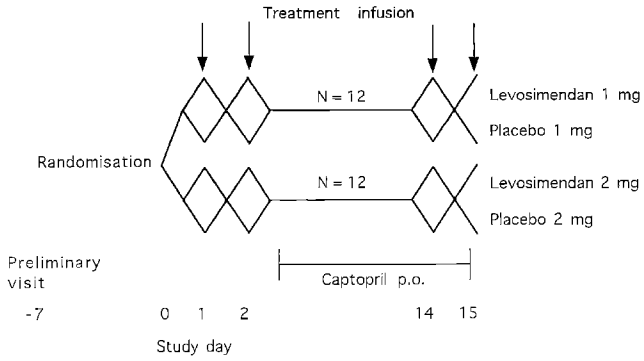

Fig. 1 The Flow chart of the study

Patients

Twenty four male patients (age < 70 y) with stable congestive heart failure of NYHA II-III functional class, with ischaemic heart disease as an aetiological factor, were recruited for the study (12 patients for each dose level). Their left ventricular ejection fraction was required to be under 40 % and left ventricular end diastolic diameter (LVEDD) > 56 mm as determined by M-mode, echocardiography within 2 weeks prior the study. The patients were required on entry to have been in sinus rhythm for at least 3 months prior to enrolment and not to have frequent extrasystolies or other arrhythmias in the ambulatory ECG. Patients with recent myocardial infarct or with instable ischaemic heart disease were excluded. If systolic blood pressure was < 100 mmHg or > 200 mmHg, or if diastolic blood pressure was < 70 mmHg or > 100 mmHg, the patient was excluded from the study.

Concomitant medication

The patients were allowed to take digoxin, ASA, β-adrenoceptor blockers, long-acting nitrates and diuretics throughout the study. Long-acting nitrates and diuretics were withdrawn on the study days. ACE-inhibitor treatment in the 2 weeks prior to the study was not allowed. Patients requiring calcium antagonists, antiarrhythmic drugs, oral anticoagulants or other vasodilatory drugs than ACE-inhibitors were excluded.

Screening

A medical examination, including medical history and clinical examination, was performed within 2 weeks before administration of the drugs. The stability of the illness was defined on the grounds of patient history, clinical examination and the stability of medication during previous 3 months. The patients were required to be able to reach a heart rate of over 100 beats · min^{-1} without ischaemic symptoms (also during treatment with β-adrenoceptors blockers). If the investigators were not sure whether the patients met these criterion, an exercise test was performed.

At the prestudy visit, resting heart rate, blood pressure and 12-lead ECG were recorded, and safety laboratory parameters were determined. The patients underwent 24 h ambulatory electrocardiogram (Holter monitoring) in order to record the baseline 24 h ECG. Ejection fraction was determined by M-mode echocardiog-

raphy (according to the Simpson rules). The study physician checked the results of the screening tests and only included patients who did not deviate from inclusion and exclusion criteria.

Study

The patients came to the Estonian Heart Centre in the evening prior to the actual study days (Days 0 and 14). The patients stayed in hospital for two days during the treatment periods. The patients fasted over both nights (starting 12 PM) when staying in hospital.

In the morning on study days, a light breakfast was served at 07.00 h. The patient drank 500 ml tap water at 08.00 h. On Days 14 and 15, they also took the morning dose of captopril at 08.00 h. Thereafter the patient lay down. On the first study days (Days 1 and 14) an intravenous cannula was inserted in to an upper arm vein in both arms, one for blood sampling and the other for infusion of the drug. Physiological NaCl solution was slowly infused through the cannula to keep the cannula open. After a minimum of 10 min in the supine position, and 1 h after the intake of captopril, the study drug (levosimendan 1.0, 2.0 mg or placebo) was infused IV. The drug infusion end time was defined as the 0 h time point. From this time onwards the patient was kept supine position for 2 h.

Two hours after drug infusion, the patient was allowed to stand up. After the last measurement of haemodynamic parameters (4 h), the patients were served a routine hospital lunch. Dinner and an evening snack were served according to the normal hospital routine.

Blood and urine samples for determination of safety parameters were taken on the second study days (Days 2 and 15) during both treatment periods. The patients were allowed to leave hospital 24 h after termination of the last infusion, if no adverse events requiring follow-up had appeared.

A blood sample for measurement of serum ACE activity was taken on Day 14 before the third infusion. ACE activity was determined in the Central Laboratory of the Helsinki University Central Hospital, Helsinki, Finland by photometric analysis of the hydrolysis (decrease in absorbance) of an ACE substrate.

Echocardiography

Two-dimensional (2-D) echocardiography was performed during the study to measure cardiac performance.

Using a standardised ultrasonographic technique and aparatus (Toshiba SAL-38 D), echocardiography was done under controlled conditions. The tracing was located at the level of the optimal view (always at the same level for the same patient), where the transducer was perpendicular to the wall structures. Echocardiography was recorded with the patient in the left oblique position. The recordings were adjusted to the rhythm of the ECG. The measurements were made 20 min and 5 min before the drug infusion and 10 min, 30 min, 2 h and 4 h after it.

The following parameters were determinated: EDD (left ventricular end-diastolic diameter), ESD (left ventricular end-systolic diameter), MRR (mean relaxation rate), FS (fractional shortening), EDV (end-diastolic volume), ESV (end-systolic volume), SV (stroke volume), CO (cardiac output), and EF (ejection fraction).

The measurements were recorded on paper for archiving. At each time point, the mean of five consecutive beats was used for analysis of the results.

Blood pressure

Systolic and diastolic blood pressures were always measured in the same arm by an automatic blood pressure manometer (Omron Automatic HEM-705-CP), with the patient supine after 10 min rest, and with an accuracy of 2 mmHg. Blood pressure was measured 20 min and 5 min before the drug infusion and 10 min, 30 min, 2 h, 4 h and 24 h after it.

Heart rate and rhythm

Heart rate was measured from the ECG recordings 20 min and 5 min before the drug infusion and 10 min, 30 min, 2 h, 4 h and 24 h after it.

Cardiac rhythm was monitored on an oscilloscope from −20 min to 2 h after study drug infusion for safety reasons. ECG leads III and V_5 were recorded at −20 min, −5 min, 10 min, 30 min, 2 h, 4 h and 24 h. If symptomatic ischaemia occurred, a paper strip was immediately recorded. If that record showed ST-segment depression of > 0.1 mV, a twelve-lead ECG was recorded on paper.

The ambulatory ECG was recorded continuously using portable Holter recorders (Marquette) from about 1 h before the infusion of 24 h after it. The number of ventricular and supraventricular extrasystoles was calculated and all dysrhythmias and conduction defects were noted. The Holter recordings were analysed at the Estonian Heart Centre.

Pharmacokinetics

In the first and second treatment periods (Days 1, 2, 14 and 15), 5 ml blood for determination of levosimendan in plasma was taken from an upper arm vein at the following time points: 0-sample at −20 min preinfusion, 0 min, 5 min, 10 min, 20 min, 30 min, 45 min, and 1 h, 1.5 h and 2 h, 3 h, 4 h, 6 h, 8 h and 24 h postinfusion.

The total amount of blood taken for pharmacokinetic determinations during the study was about 450 ml. The samples were stored at −20°C until analysed. The levosimendan concentration in plasma was determined at the Chemical Research Department of Orion-Farmos by HPLC.

The pharmacokinetic parameters of levosimendan were calculated using a noncompartment model by the PCNONLIN computer program (version 4.0B, model 202). The program estimated the terminal elimination rate constant. Terminal half-life ($t_{1/2el}$) was calculated by dividing ln2 by the corresponding rate constant. Area under curve (AUC) was calculated by the trapezodial rule from the beginning of the infusion to the last measurable concentration and was extrapolated to infinity. C_{max} was determined from the observed concentration data. Mean residence time (MRT) was extrapolated to infinity.

Safety

Safety during the study was followed by laboratory tests, ECG monitoring and blood pressure and heart rate recordings. All signs and symptoms observed during the study days were recorded using active inquiry.

The following laboratory tests were done within 2 weeks before and 24 h postinfusion on Days 3 and 16 (B = blood, S = serum, U = urine): B-Haemoglobin (B-Hb), B-Leukocytes (B-Leuk), B-Thrombocytes (B-Thromb), B-Haematocrit (B-Hcr), B-Erythrocytes (B-Erythr), S-Sodium (S-Na), S-Potassium (S-K), S-aspartate aminotransferase (S-ASAT), S-alanine aminotransferase (S-ALAT), S-alkaline phosphatase (S-APHOS), S- Creatinine (S-Creat), S-gamma glutamyl transferase (S-gamma-GT), S-Glucose (S-Gluc), U-protein (U-prot) and U-glucose (U-gluc).

The adverse event inquiry was made before and 24 h after each drug infusion.

Statistical methods

The primary pharmacodynamic responses 10 min after infusion, blood pressure, heart rate, ejection fraction etc., were analysed separately by an univariate repeated measures analysis of variance model, which included both random and fixed effects (so called mixed model). The model also included two-factor interactions: period and day, period and medication, day and medication, group and period, group and day, group and medication. Interaction could be seen as a measure of the nonadditivity of the main effects. The most important two-factor interaction in the study was the interaction between period and medication, which was the simple factor for statistically evaluating the pharmacodynamic interaction between levosimendan and captopril.

Pharmacokinetic parameters were analysed applying the same statistical method.

Analyses of variance were performed using the MIXED-procedure of the SAS-system, which also produced the estimates and confidence intervals presented in the analysis of variance tables.

Change was used as a response variable for the laboratory safety parameters, which were measured before the study (at the screening visit) and 24 h after infusion. Average, minimum and maximum changes were calculated and 95% confidence intervals were estimated.

In testing hypotheses the conventional level of 5% was regarded as the level of statistical significance.

Results

Patient characteristics and eligibility for analysis

The mean age of the patients were 56 y (range 34–66 y), mean weight was 79 kg (range 67–105 kg) and mean height was 173 cm (range 164–187 cm). The NYHA classification was II in 21 patients and was III in 3 patients. The mean ejection fraction of the patients at the screening visit using M-mode echocardiography was 33%.

Twenty two patients completed the study according to the protocol. One patient discontinued the study after the 14th study day, after receiving placebo, because of increased chest pain. Another patient had myocardial infarction and ventricular fibrillation, and died on the 15th study day just before the last round of study drug administration. In addition to those patients, pharmacokinetic calculations (elimination half life, MRT and AUC) could not be performed in eight further cases (out of the total of 48 rounds of levosimendan) due to missing samples, mistakes in sampling or deviant plasma concentrations (samples taken from the drug infusion canula).

Blood pressure and heart rate

Administration of captopril significantly decreased systolic −7 (2) mmHg ($P = 0.0088$) and diastolic −4 (2) mmHg blood pressures (mean (SEM) $P = 0.0112$) (Table 1). Levosimendan had no effect on systolic (1 (2) mmHg, $P = 0.5206$) or diastolic (−2 (1) mmHg, $P = 0.1008$) blood pressure. The interaction [effect] between levosimendan and captopril on systolic and diastolic blood pressure was not significant.

Table 1 Blood pressure (BP) (mmHg) and heart rate (HR) (beats · min^{-1}) after levosimendan (LS) and captopril. Mean with (SEM)

Treatment	Parameter	Time −20 min[a]	−5 min	10 min	30 min	2 h	4 h	24 h
LS 1 mg ($n = 12$)	syst BP	121 (5)	120 (5)	117 (4)	119 (5)	115 (5)	119 (5)	118 (5)
	diast BP	78 (2)	77 (2)	78 (2)	76 (3)	70 (3)	75 (3)	76 (2)
	HR	63 (2)	64 (2)	62 (2)	61 (2)	60 (2)	66 (3)	62 (2)
LS 1 mg + captopril ($n = 11$)	syst BP	108 (5)	109 (5)	110 (4)	110 (4)	111 (4)	115 (4)	117 (5)
	diast BP	71 (2)	73 (3)	72 (2)	69 (2)	72 (2)	74 (3)	75 (3)
	HR	62 (3)	61 (3)	61 (2)	61 (2)	61 (2)	61 (4)	63 (3)
placebo 1 mg ($n = 12$)	syst BP	125 (6)	125 (6)	119 (6)	127 (9)	119 (6)	119 (5)	120 (6)
	diast BP	80 (3)	83 (3)	79 (3)	81 (4)	78 (3)	76 (3)	75 (3)
	HR	61 (2)	61 (3)	64 (2)	63 (3)	64 (3)	66 (3)	65 (3)
placebo 1 mg + captopril ($n = 11$)	syst BP	115 (4)	112 (4)	110 (4)	111 (4)	117 (5)	115 (4)	111 (5)
	diast BP	78 (3)	74 (3)	75 (3)	75 (3)	77 (3)	77 (3)	75 (2)
	HR	65 (3)	60 (3)	59 (2)	61 (2)	61 (3)	63 (2)	62 (2)
LS 2 mg ($n = 12$)	syst BP	130 (5)	129 (6)	131 (6)	125 (4)	128 (4)	123 (4)	123 (5)
	diast BP	82 (5)	81 (5)	78 (5)	74 (5)	76 (4)	75 (4)	78 (3)
	HR	63 (3)	66 (3)	65 (4)	64 (3)	61 (3)	61 (3)	65 (3)
LS 2 mg + captopril ($n = 12$)	syst BP	125 (5)	126 (5)	122 (5)	120 (3)	122 (3)	123 (4)	126 (5)
	diast BP	73 (4)	74 (4)	72 (4)	69 (4)	73 (3)	74 (3)	73 (4)
	HR	58 (2)	62 (3)	64 (3)	62 (3)	58 (2)	64 (2)	61 (2)
Placebo 2 mg ($n = 12$)	syst BP	133 (5)	129 (6)	125 (6)	125 (6)	125 (5)	125 (4)	128 (5)
	diast BP	79 (4)	81 (4)	80 (4)	77 (4)	75 (5)	76 (3)	79 (5)
	HR	64 (2)	65 (2)	63 (3)	59 (2)	61 (3)	60 (3)	64 (3)
Placebo 2 mg + captopril ($n = 11$)	syst BP	124 (5)	122 (5)	119 (4)	121 (4)	123 (4)	122 (3)	126 (4)
	diast BP	74 (4)	74 (4)	73 (3)	73 (4)	75 (3)	73 (3)	73 (3)
	HR	58 (2)	57 (2)	57 (2)	55 (2)	59 (3)	58 (2)	61 (2)

[a] The determinations were made 20 and 5 min before drug infusion and 10 min, 30 min, 2 h, 4 h and 24 h after it.
[b] Mean with (SEM)

Supine heart rate was decreased by −3 (1) (mean SEM) beats · min^{-1} after captopril treatment, which was statistically significant ($P = 0.0329$). Levosimendan increased supine heart rate 2 (1) beats · min^{-1} ($P = 0.1632$), which was not statistically significant. The estimated interaction effect was not significant (4 (2) beats · min^{-1}, $P = 0.1191$).

Echocardiographic measurements

The mean left ventricular end diastolic diameter (LVEDD) at 10 min did not change after levosimendan 1 mg, nor did the mean left ventricular diastolic volume (LVEDV; data not shown), nor was any change after 2 mg levosimendan. Concomitant captopril did not affect the result.

Levosimendan 1 mg decreased the mean left ventricular systolic volume (LVESV) from 133 ml to 126 ml, whereas left ventricular end systolic diameter (LVESD) did not change (data not shown). Levosimendan 2 mg did not change LVESV or LVESD. The effects of captopril, levosimendan and their interaction on left ventricular systolic diameter and volume were not statistically significant.

In the main study 2-D echocardiography was used unlike the screening visit when the M-mode echocardiography was used. Therefore, higher ejection fraction values compared to screening were obtained. After levosimendan 1 mg mean FS increased from 22% to 24% compared to placebo, and mean EF 43% to 46% at 10 min (data not shown). The mean FS remained at 23% and mean EF decreased just slightly from 45% to 44% compared to placebo 10 min after levosimendan 2 mg. After captopril treatment, levosimendan 1 mg or 2 mg did not change the mean FS or mean EF. The effects of captopril, levosimendan and their interaction on fractional shortening or ejection fraction were not statistically significant. There was no significant change in MRR (data not shown).

The effect of treatment with 1 mg levosimendan on SV was statistically significant ($P = 0.047$), with a mean increase of 10 ml (95% CI: 0.1–19.7 ml) at 10 minutes (Table 2). Cardiac output was increased after levosimendan (1 or 2 mg) in comparison to placebo by 0.5 l ($P = 0.0043$) at 30 min after the drug infusion, and by 0.4 l ($P = 0.014$) when 10 and 30 min observations were combined. The change in CO after 10 minutes was not statistically significant ($P = 0.15$).

Ambulatory ECG recordings

The mean heart rate in the Holter-records (24 h) was 65 (2) beats · min^{-1} (mean (SEM) after both levosimendan 1 mg and placebo, and 64 (3) beats · min^{-1} after levosimendan 2 mg and placebo. With concomitant captopril treatment, the heart rate was 67 (2) beats · min^{-1} after levosimendan 1 mg and 66 (2) beats · min^{-1} after placebo. After levosimendan 2 mg the heart rate was 62 (2) beats · min^{-1} and after placebo with concomitant captopril treatement it was 63 (2) beats · min^{-1}. Neither levosimendan 1 mg nor 2 mg had any effect on mean heart rate.

All the patients had some ventricular ectopic beats recorded during the screening visit. After placebo the number of VEs was 85 (29) per hour and the number of runs of VTs was 4 (2) (Fig. 2). After levosimendan 1 mg the corresponding figures were 79 (31) and 5 (2). After levosimendan 2 mg the number of VEs was 22 (6) and the number of runs or VTs was 2 (1). The numbers of VEs and VTs after placebo were 36 (12) and 4 (2). With concomitant captopril treatment the number of VEs after placebo was 86 (38) per hour and the number of runs or VTs was 3 (2). After

Table 2 Echocardiographic parameters after levosimendan (LS) and captopril. Mean with (SEM)

Treatment	Parameter	Time −20 min	−5 min	10 min	30 min	2 h	4 h
LS 1 mg ($n = 12$)	SV (ml)	99 (7)	97 (8)	107 (11)	102 (8)	101 (10)	102 (8)
	CO (l · min^{-1})	6.0 (0.4)	6.0 (0.4)	6.6 (0.6)	6.2 (0.4)	6.4 (0.5)	6.7 (0.5)
Placebo 1 mg (n = 12)	SV (ml)	98 (10)	90 (8)	99 (9)	96 (7)	100 (9)	103 (8)
	CO (l · min^{-1})	5.8 (0.6)	5.3 (0.4)	6.0 (0.5)	5.8 (0.4)	5.7 (0.4)	6.3 (0.3)
LS 1 mg + captopril ($n = 11$)	SV (ml)	106 (9)	97 (9)	103 (9)	103 (8)	100 (8)	98 (8)
	CO (l · min^{-1})	6.1 (0.5)	5.9 (0.5)	6.3 (0.5)	6.3 (0.4)	6.1 (0.5)	6.2 (0.6)
Placebo 1 mg + captopril ($n = 11$)	SV (ml)	100 (7)	104 (9)	99 (7)	103 (9)	103 (8)	108 (10)
	CO (l · min^{-1})	6.9 (0.8)	6.1 (0.6)	6.0 (0.5)	6.1 (0.6)	6.0 (0.5)	6.7 (0.6)
LS 2 mg ($n = 12$)	SV (ml)	98 (7)	105 (6)	102 (7)	100 (8)	103 (8)	105 (7)
	CO (l · min^{-1})	6.3 (0.5)	6.7 (0.5)	6.5 (0.4)	6.2 (0.5)	6.2 (0.4)	6.2 (0.4)
Placebo 2 mg ($n = 12$)	SV (ml)	104 (8)	102 (10)	105 (6)	103 (7)	102 (6)	100 (6)
	CO (l · min^{-1})	6.3 (0.5)	6.5 (0.5)	6.4 (0.3)	6.0 (0.5)	6.2 (0.4)	6.2 (0.4)
LS 2 mg + captopril ($n = 12$)	SV (ml)	106 (7)	102 (7)	103 (7)	112 (7)	105 (7)	110 (7)
	CO (l · min^{-1})	6.1 (0.3)	6.2 (0.3)	6.3 (0.4)	6.9 (0.3)	6.1 (0.5)	6.4 (0.3)
Placebo 2 mg + captopril ($n = 11$)	SV (ml)	106 (8)	105 (8)	108 (9)	106 (9)	106 (8)	109 (8)
	CO (l · min^{-1})	5.9 (0.4)	5.7 (0.4)	6.1 (0.6)	5.7 (0.4)	5.8 (0.5)	6.4 (0.5)

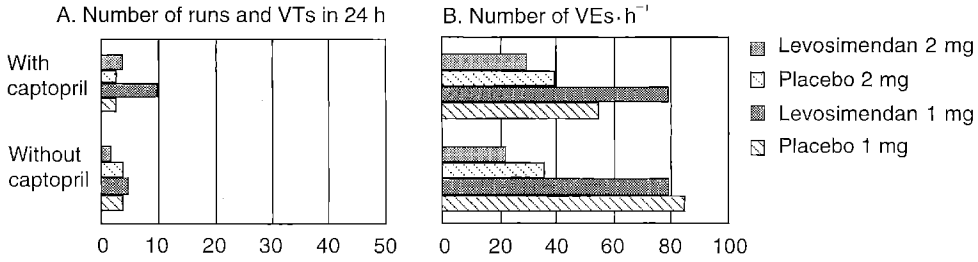

Fig. 2 **A** The frequency of runs (3 or more consecutive ventricular ectopic beats) and ventricular tachycardias (VTs) (5 or more consecutive ventricular ectopic beats) in 24-hour ambulatory ECG recording after 1 or 2 mg levosimendan or placebo. **B** The number of ventricular ectopic beats (VEs) during the ambulatory ECG recording

Table 3 Pharmacokinetic parameters after levosimendan (LS) and captopril. Mean with (SEM)

Treatment	Parameter C_{max} (ng · ml^{-1})	$AUC^{0-\infty}$ (ng × h · ml^{-1})	$t_{1/2el}$ (h)	MRT (h)
LS 1 mg	47.6 (4.2) (n = 11)	44.0 (4.7) (n = 9)	0.8 (0.1) (n = 9)	1.0 (0.1) (n = 9)
LS 1 mg + captopril	49.6 (4.0) (n = 11)	46.2 (3.6) (n = 9)	0.8 (0.1) (n = 9)	1.1 (0.1) (n = 9)
LS 2 mg	94.4 (10.9) (n = 10)	96.4 (12.6) (n = 10)	1.0 (0.1) (n = 10)	1.3 (0.1) (n = 10)
LS 2 mg + captopril	108.2 (13.1) (n = 11)	89.3 (12.2) (n = 9)	0.7 (0.1) (n = 9)	0.9 (0.1) (n = 9)

levosimendan 1 mg these figures were 79 (30) and 10 (6). After levosimendan 2 mg the mean number of VEs was 30 (11) and the number of runs or VTs was 4 (1) with concomitant captopril treatment. The numbers after placebo were 40 (24) and 3 (1) respectively.

Pharmacokinetics

The terminal elimination half-life could not be determinated in all the patients because there were few data points during the terminal elimination phase. There was no significant change in the mean pharmacokinetic parameters of levosimendan 1 mg after concomitant treatment with captopril (Table 3). After 2 mg levosimendan, the mean AUC was somewhat larger and mean $t_{1/2el}$ and MRT were longer than after concomitant treatment with captopril, but there were no significant differences between these parameters.

Safety

One serious adverse event was reported during the study. One patient died about 24 h after administration of levosimendan 2 mg with concomitant captopril treatment. The cause of death was acute myocardial infarction according to the autopsy report. No levosimendan was found in plasma samples taken 18 h before death. The patient did not have a fall in blood pressure or an arrhythmia after the drug infusion on the previous day and no causality between the study drug and patient death could be established. Another patient reported cardiac pain on Days 8, 11 and 14 of the study and discontinued it before receiving levosimendan. Certain other adverse events were reported during captopril therapy before the subjects received levosimendan. These include a dazed feeling (3 patients), tiredness (2 patients), fall in diastolic blood pressure (2 patients), one case of dizziness and one case of palpitation.

One patient had a clinically significant increase in serum potassium from a baseline value of 3.80 to 5.85 mmol · l^{-1} on the fifth study day. Three other patients had potassium values exceeding the reference range. These changes could be considered as clinically significant and were attributed to the captopril treatment. One of those patients also had an increase in serum creatinine on the 16th study day, from 116 to 178 μmol · l^{-1}.

Discussion

Levosimendan is an inodilator drug intended for the treatment of heart failure. Previous clinical experience of inodilator drugs is not encouraging. Phosphodiesterase inhibitors have increased mortality both in patients not treated with ACE inhibiting drugs [11] and in patients using them [12]. Recently, however, interesting results have been obtained with vesnarinone, a PDE III inhibiting drug with sodium channel blocking properties [13]; a low dose (60 mg per day) did reduce mortality, whereas high doses (120 mg per day) reduced survival. The low dose was devoid of marked haemodynamic effects and did not increase the heart rate.

Levosimendan, in addition to its calcium sensitising activity, is also a PDE inhibitor. However, after the 1 or 2 mg doses (given as a bolus infusion in 10 min) no

increase in heart rate was observed which might have indicated a phosphodiesterase inhibition-induced increase in cAMP. This is in accordance with data from studies of guinea-pig Langendorff hearts, in which no increase in cAMP could be detected at comparable drug levels [14]. At these concentrations, too, the effect of the drug on stroke volume was modest. From previous studies it is known, however, that a single intravenous dose of the drug considerably reduces preload [6]. In that study, pulmonary wedge pressure was reduced by 40–50% in NYHA II-III patients after an IV, dose of 1–2 mg (given as a bolus in 5 min), whereas SV was increased by 15% by lower dosages (0.25–0.5 mg) but not after higher doses. The reduced filling will result in reduced stroke volume and this could well explain the fact that no change was seen in SV or cardiac output, especially after concomitant administration of levosimendan and captopril. It should also be remembered that echocardiography has limited accuracy and repeatability when a diseased heart is evaluated, especially if there are scars of previous myocardial infarcts as in our patients. Therefore, these results must be taken cautiously. However, the design of the study included repeated haemodynamic measurements after different phases. The use of invasive haemodynamic measurements was not acceptable in our relatively asymptomatic patients.

Captopril has shown to be beneficial in the long-term treatment of heart failure [15] and ACE-inhibitors are now considered as one of the primary therapies for moderate and severe heart failure [16]. After single doses of an ACE inhibitor, the typical haemodynamic changes are an increase in SV and cardiac index and a decrease in PCWP [17, 18]. The initial haemodyamic effect of ACE-inhibitors changes during the maintenance therapy; after 2 weeks treatment with captopril, the initial increase in cardiac index is attenuated, but the increase in stroke volume and decrease in filling pressure as well as in heart rate are maintained [18]. In our study, there was a tendency to improved SV after captopril treatment. However, the method of evaluation was too rough to determine the possible change.

Even though new inodilator drugs in clinical trials are used concomitantly with the conventional heart failure therapy of diuretics, ACE inhibitors and digitalis, there are few published interaction studies. Drug interactions, should be taken seriously, however, since typical heart failure patients are old, they may have other diseases affecting the drug elimination and they receive multiple drugs, often for other concomitant diseases. Excessive vasodilatation could lead to hypotension and myocardial ischaemia with rhythm disorders. There were no signs of hypotension in our study and no adverse effects were reported during or immediately after the drug infusions. Levosimendan has been well tolerated in previous trials in heart failure patients. Vasodilatory adverse effects (headache, dizziness, flushing) have been occasionally reported, but the drug has been devoid of proarrhythmic effects [19].

In conclusion, no additional decrease in blood pressure was seen when levosimendan was added to captopril, and no change in heart rate was observed. Cardiac output and stroke volume tended to improve in patients receiving levosimendan. Intravenous levosimendan and captopril can safely be combined in the treatment of heart failure patients.

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