Eur J Clin Pharmacol (1994) 47: 267–274

PHARMACODYNAMICS

J. Lilleberg · S. Sundberg · M. Häyhä · J. Akkila
M. S. Nieminen

Haemodynamic dose-efficacy of levosimendan in healthy volunteers

Received: 25 February 1994/Accepted in revised form: 27 June 1994

Abstract Levosimendan is a new calcium-sensitiser intended for the treatment of congestive heart failure. The results of preclinical studies indicate it has positive inotropic and vasodilator effects. In the open study reported here up to 5 mg levosimendan and vehicle were administered to 8 healthy male volunteers at one- to 2-week intervals. Efficacy was evaluated using M-mode echocardiography, and by measuring systolic time intervals, recording ECG and measuring blood pressure.

For almost all haemodynamic parameters except heart rate (HR) the maximum effect was seen 10 or 20 min after drug infusion. Effects 10 min after infusion of drug and vehicle were compared.

HR was significantly increased 10 min only after infusion of 5 mg: significant increases were seen 60 min after infusions of 2, 3 and 5 mg (4, 8 and 17 beats · min^{-1}, respectively).

Diastolic blood pressure was significantly lower after doses of 1 mg or more. The decrease after 5 mg was 17 mm Hg. Systolic blood pressure tended to increase. Fractional shortening (FS) and ejection fraction (EF) increased significantly after doses of 1 mg and higher. EF 10 min after infusion of vehicle was 54 %. It increased to 73 % after 5 mg. Decreases in electromechanical systole ($QS2_i$) 10 min after 2, 3 and 5 mg were significant. There were no clinically significant adverse events or changes in laboratory safety values.

The changes in QS2i, FS, EF and blood pressure indicate that levosimendan has positive inotropic and vasodilator effects in healthy subjects.

Key words Levosimendan, Calcium sensitiser; echocardiography, systolic time intervals, healthy volunteers, adverse events

J. Lilleberg (✉) · M. S. Nieminen
Cardiovascular Laboratory, First Department of Medicine, Helsinki University Central Hospital, FIN-00290 Helsinki, Finland

S. Sundberg · M. Häyhä · J. Akkila
Orion-Farmos Pharmaceuticals, Espoo, Finland

Calcium sensitisers belong to a new group of drugs intended for treatment of congestive heart failure. Theoretically their mode-of-action should be very favourable as they sensitise contractile proteins to calcium ions, and the contractile force of myocardial cells increase. In addition, proarrhythmic effects and increased use of energy by free calcium ions can be prevented [1, 2]. However, calcium sensitisation may retard relaxation of myofibrils [3].

Levosimendan is the active (–) enantiomer of simendan, a new calcium sensitiser. Levosimendan is bound calcium dependently to cardiac troponin C, which causes its inotropic effects. It has been suggested that it stabilises the calcium-induced change in troponin conformation [4, 5]. The results of in vitro studies have shown that this calcium-dependent sensitization results in an inotropic effect without impairment of relaxation of myofibrils [6].

Simendan also selectively inhibits phosphodiesterase III (PDE III) purified from human or guinea-pig cardiac tissue [7, 8]. However, at a clinically relevant level of inotropy (a 25–30 % increase in the pressure derivative of the left ventricle), levosimendan did not significantly increase phosphorylation of contractile proteins, or the concentration of cyclic adenosine monophosphate in perfusion guinea-pig hearts. In contrast, the reference compounds pimobendan and EMD 53998 significantly increased these parameters. These observations suggest that the positive inotropic effect of levosimendan is mainly related to calcium sensitization. In contrast, PDE inhibition also contributes to inotropic responses of pimobendan and EMD-5399 [9].

In patch clamp recordings, simendan decreased inward calcium current [10, 11]. Simendan has also been shown to increase coronary flow, even through partly ligated coronary arteries, and to improve total and segmental contractile function in a model of acute heart failure produced by severe regional ischaemia in dogs [11].

The haemodynamic effects of simendan in healthy volunteers have been studied using echocardiography.

Left ventricular function was significantly improved in a dose-dependent manner. Statistically significant haemodynamic effects were observed after doses of 0.5–1 mg or more. The mean ejection fraction (EF) 10 min after infusion of 5 mg was increased from 56 to 69 %, and mean maximal velocity of circumferential fibre shortening (VCF_{max}) increased by 36 %, while heart rate (HR) was increased by only 8 beats · min^{-1}. Maximum relaxation rate tended to increase. No change in ECG was observed [12].

Because all of the pharmacological effects of simendan are predominantly attributable to levosimendan it was considered important to evaluate the haemodynamic effects of levosimendan in healthy volunteers, using echocardiography and by measuring systolic time-intervals. Because it represented the first time levosimendan had been administered to man, the study was open, and a vehicle infusion was used as the control.

Materials and methods

The study group comprised eight healthy male volunteers selected from a larger group. The eight volunteers were aged 23–29 y (mean 25 y), their mean body weight was 78 (5.3) kg (SD) and height 185 (3.4) cm (SD). Results of routine safety laboratory tests lay within normal ranges. The subjects were considered healthy on the basis of history, results of physical examination, resting ECG and baseline echocardiographic findings. Particular attention was paid to obtaining good echocardiographic images and to excluding subjects with left ventricular asynergy.

The experiments were done following an overnight cast. To avoid dehydration and consequent low preload, subjects drunk 200 ml juice, after which they lay semisupine for 25–30 min before the first recordings. They then received successive 5 min infusions of isotonic saline and levosimendan. Blood samples for the measurement of drug concentration were taken from the contralateral arm. Echocardiographic recordings and blood pressure measurements were undertaken simultaneously, 20 and 10 min before and 0, 5, 10, 20, 30, 60 and 120 min after completion of infusion. Systolic time intervals were recorded 10 min before and 0, 10 and 60 min after the infusion. During the first study day, subjects received the vehicle infusion. On the following study days, always one week apart, they received 0.2, 0.5, 1, 2, 3 and 5 mg levosimendan, in that order.

The study protocol was approved by the Ethical Committee of the First Department of Medicine, Helsinki University Central Hospital. Written informed consent had been given by all subjects. The study followed the guidelines of the Declaration of Helsinki.

Blood pressure (BP) was conventionally measured in the right arm with a sphygmomanometer. Heart rate was measured from the ECG trace on the echocardiography recordings. Ambulatory ECG monitoring started approximately 50 minutes before, and stopped 8 hours after each infusion. Heart-rate-corrected QT intervals (QT_c) were derived by dividing the measured QT intervals by the square root of measured RR intervals [13].

The subject lay semisupine, in the left lateral oblique position. A Toshiba SSH-160A echocardiograph attached to a line scan recorder LSR-100A and a 2.5 MHz transducer with dynamic focus was used. ECG lead II traces and phonocardiograms were recorded simultaneously on 6-inch thermal paper, at a speed of 100 mm · s^{-1}. M-mode recordings were guided by means of simultaneous two-dimensional imaging. The left ventricular internal diameters were measured just below the tip of the anterior mitral leaflet.

Echocardiograms were analysed semi-automatically using an X–Y digitizer in accordance with the method of Upton and Gibson [14], and a Digital PDP 11/34 minicomputer. Left ventricular end-diastolic diameter (LVEDD) was measured at the beginning of the Q-wave, and the left ventricular end-systolic diameter (LVESD) was measured at the first high frequency deflections of the second heart sound [15]. Fractional shortening (FS) was calculated using the formula:

$$FS\,(\%) = \frac{LVEDD - LVESD}{LVEDD} \times 100$$

Left ventricular end-diastolic and end-systolic volumes were calculated using the Teichholz formula [16]. Stroke volume (SV) and ejection fraction (EF) were calculated from left ventricular volumes in the standard way. Cardiac output (CO) was calculated by multiplying HR by SV. Maximal velocity of circumferential fiber shortening (VCF_{max}) and maximal relaxation rate (MRR) were automatically calculated by the X–Y digitizer program.

Total peripheral vascular resistance (TPR) was calculated using the formula:

$$TPR\,(dyn \times s \cdot cm^{-5}) = \frac{mBP}{CO} \times 80$$

where mBP stands for mean blood pressure, calculated in the standard way.

Average echocardiographic variables were calculated from three consecutive or almost consecutive cycles at the end of expiration.

A microphone was placed over the upper precordium in the best position for recording initial high-frequency vibrations of the first and second heart sounds. Recordings were made with a Toshiba HSM-05A microphone (frequency range 20–600 Hz ± 6 dB time constant ± 3.1 ms).

Carotid arterial pulsation was recorded using a Toshiba TPW-01A pressure sensor (resonance frequency 1 kHz, time constant 1.8 s at low frequency), which measured static and dynamic relative displacement. Simultaneous recordings of carotid arterial pulsation, phonocardiogram and ECG were undertaken with the head of the subject tilted towards the contralateral shoulder, using the line-recorder at speed of 100 mm · s^{-1}.

Electromechanical systole (QS2) was measured from the beginning of the Q-wave in the ECG to the beginning of the aortic component of the second heart sound (AII). Left ventricular ejection time (LVET) was measured from the beginning of the upstroke of the carotid pulse curve to its incisure. Pre-ejection period (PEP) was calculated as QS2-LVET [17]. QS2 was corrected for heart rate using the formula $QS2_i = QS2 + 2.1 \times HR$ [18], PEP was not heart-rate-corrected [19, 20].

The repeatability of the digitisation procedure was assessed by taking 20 recordings at random and blindly digitising them twice, in random order.

The within-day repeatability of echocardiographic recordings was evaluated by comparing the results of the recordings from 7 subjects 20 and 10 min before completion of six drug infusions (0.1–5.0 mg). Because only one recording of systolic time intervals was undertaken before the infusions, evaluation of their within-day repeatability was not possible.

The primary responses (CO, EF, HR etc.) were analysed separately using a univariate repeated measures analysis of variance model, with dose or time relative to dosing being treated as a within-subject factor. Instead of simple P-values, the results of statistical analysis are reported using the more illustrative simultaneous 95 % confidence intervals (in brackets), which were constructed by applying critical values from the corresponding Dunnett's tests.

In analyses of the duration of the effect, baseline values of measurements 10 min before dosing were compared with the findings 0 min–2 h after dosing. Numbers of subjects included in time-comparison analysis were 8 after 0.2–2 mg, 7 after 3 mg and 5 after 5 mg.

Table 1 Repeatability of haemodynamic, echocardiographic and systolic time interval measurements

	Repeatability of digitisation[a]	Within day repeatability[a]	Between day repeatability[a]
HR (beats · min^{-1})	0.5	6.1	15.2
sBP (mmHg)	–	13.0	19.0
dBP (mmHg)	–	8.0	13.2
LVESD (mm)	0.9	2.4	2.9
LVEDD (mm)	1.6	2.6	3.4
FS (%)	2.2	3.7	4.7
EF (%)	3.0	5.3	6.8
VCF_{max} (lenghts · s^{-1})	0.43	0.42	0.49
MRR (mm · s^{-1})	23.6	28.2	39.1
CO (l · min^{-1})	0.53	0.82	1.29
TPR (dyn × s · cm^{-5})	168	288	536
QS2 (ms)	17.0		32.7
LVET (ms)	15.2		28.7
PEP (ms)	27.4		30.7
PEP/LVET	0.094		0.111

HR, Heart rate; sBP, systolic blood pressure; dBP, diastolic blood pressure; LVESD, left ventricular end-systolic diameter; LVEDD, left ventricular end-diastolic diameter; FS, fractional shortening; EF, ejection fraction; VCF_{max}, maximal velocity of left ventricular circumferential fiber shortening; MRR, maximal relaxation rate; CO, cardiac output; TPR, total peripheral vascular resistance; QS2, electromechanical systole; LVET, left ventricular ejection time; PEP, pre-ejection period; PEP/LVET, the relation between pre-ejection period and left ventricular ejection time

[a] The repeatability coefficients show the estimated difference between two measurements at the probability level of 95 %

In dose-comparison analysis, responses 10 min after each dose were compared to the control level of measurements 10 min after administration of vehicle. To avoid possible bias from the dropouts, the data from all the subjects were utilised in the analysis. Minor differences between observed mean values and estimated dose-effects after doses of 3 and 5 mg were due to the imbalance caused by the dropouts.

The repeatability coefficient of digitised echocardiographic measurements was estimated using the method of Bland and Altman [21]. Within-day variation was estimated by analysis of variance, using measurements made 10 and 20 min before dosing. The upper 95 % confidence limit for differences between two measurements was also used to calculate within-day repeatability. Statistical calculations were carried out using the SAS statistical package.

Results

Repeatability

Repeatability of digitisation was satisfactory for all parameters measured. Within-day and between-day repeatability was good or satisfactory, having regard to the physiological variation evident in HR and BP values (Table 1).

Doses

All 8 subjects received 2 mg, 7 received 3 mg, and 5 of them received 5 mg. The reason in all cases for dropping-out of the study was a decrease in diastolic blood pressure to below 40 mm Hg. Effects of levosimendan in relation to time were always measured in relation to baseline value at each dose level, so the numbers of subjects differed. The maximum effect on almost all the haemodynamic parameters was observed 10 or 20 min after the drug infusion.

Results after administration of 3 mg are reported only in Table 2. They differed only minimally from results after 2 mg.

Effects of dose on heart rate and blood pressure

Mean HR was increased by 3 beats · mm^{-1} (–3, 8) after 2 mg and by 6 beats · min^{-1} (+0, 13) after 5 mg 10 min after infusion as compared to the vehicle infusion. After higher dosages the maximal increase in HR was seen at time points 30 and 60 min. Mean systolic BP was virtually unchanged up to 2 mg; 10 min after infusion it was 7 mm Hg (–3, 17) higher after 2 mg and 11 mm Hg (–1, 23) higher after 5 mg than after the vehicle (Table 2).

Four out of 5 subjects showed a decrease in diastolic BP below 40 mm Hg at least once after the 5 mg dose; it was unmeasurably low in one subject 10 min after the 5 mg infusion. Diastolic BP was lower by –5 (–13, 2), –8 (16, –0), –12 (–19, –4) and –17 mm Hg (–27, –7), respectively, after the doses 0.5, 1, 2 and 5 mg than after the vehicle (Table 2).

Effects of dose on haemodynamic parameters assessed by echocardiography

Similar responses were observed in relation to FS and EF. Both were higher after doses of 1, 2, 3 and 5 mg than after infusion of vehicle. Changes in FS after 0, 0.5, 1, 2 and 5 mg are shown in Fig. 1. Mean values of EF 10 min after infusion were 54 % after vehicle, 61 %

Table 2 Mean values with (SEM) of haemodynamic parameters 10 min after intravenous infusion of levosimendan in healthy subjects, 95 % confidence interval of changes in relation to the effect of vehicle infusion are shown in brackets

Dose	Vehicle ($n = 8$)	0.5 mg ($n = 8$)	1 mg ($n = 8$)	2 mg ($n = 8$)	3 mg ($n = 7$)[a]	5 mg ($n = 5$)[b]
HR (beats · min^{-1})	53 (3)	49 (3) (–9, 2)	50 (3) (–8, 3)	56 (3) (–3, 8)	55 (3) (–3, 8)	58 (4) (+0, 13)
sBP (mm Hg)	106 (3)	109 (3) (–8, 12)	111 (3) (–6, 14)	113 (3) (–3, 17)	115 (6) (–2, 19)	120 (4) (–1, 23)
dBP (mm Hg)	57 (2)	52 (1) (–13, 2)	49 (2) (–16, –0)	46 (2) (–19, –4)	46 (4) (–20, –3)	40 (4) (–27, –7)
LVESD (mm)	39 (1)	37 (1) (–3, 1)	37 (1) (–4, –0)	34 (1) (–7, –3)	34 (1) (–7, –3)	32 (2) (–9, –5)
LVEDD (mm)	54 (1)	54 (1) (–2, 3)	54 (1) (–2, 3)	54 (1) (–2, 3)	55 (1) (–2, 3)	55 (2) (–2, 4)
FS (%)	28 (1)	31 (1) (–0, 6)	33 (1) (1, 7)	38 (1) (7, 12)	38 (1) (7, 13)	42 (1) (10, 17)
EF (%)	54 (2)	58 (2) (1, 8)	61 (2) (3, 10)	68 (2) (10, 17)	68 (1) (10, 18)	73 (2) (14, 22)
VCF_{max} (circ/s)	2.1 (0.1)	2.3 (0.1) (–0.2, 0.5)	2.4 (0.1) (–0.0, 0.7)	3.0 (0.2) (0.5, 1.2)	3.0 (0.1) (0.6, 1.3)	3.4 (0.1) (0.9, 1.7)
MRR (mm/s)	127 (8)	126 (9) (–23, 19)	131 (11) (–18, 24)	144 (7) (–5, 37)	141 (5) (–7, 36)	164 (6) (7, 56)
CO (l/min)	4.0 (0.3)	4.1 (0.2) (–0.5, 0.7)	4.3 (0.3) (–0.3, 1.0)	5.2 (0.1) (0.6, 1.8)	5.5 (0.1) (0.8, 2.1)	6.2 (0.4) (1.5, 2.9)
TPR (dyn s cm^{-5})	1510 (110)	1420 (80) (–333, 141)	1310 (70) (–436, 38)	1050 (40) (–701, –226)	1050 (80) (–738, –220)	870 (70) (–946, –353)
QS2i (ms)	528 (6)	521 (5) (–20, 4)	518 (6) (–22, 2)	512 (7) (–28, –4)	515 (8) (–27, –2)	491 (12) (–50, –22)

SEM, Standard error of the mean; HR, heart rate; sBP, systolic blood pressure; dBP, diastolic blood pressure; LVESD, left ventricular end-systolic diameter; LVEDD, left ventricular end-diastolic diameter; FS, fractional shortening; EF, ejection fraction; VCF_{max}, maximal velocity of left ventricular circumferential fiber shortening; MRR, maximal relaxation rate; CO, cardiac output; TPR, total peripheral vascular resistance; QS2i, electromechanical systole

[a] $n = 6$ for dBP and TPR; [b] $n = 4$ for dBP and TPR

after 1 mg, 68 % after 2 mg and 73 % after 5 mg; the corresponding 95 % confidence intervals of the differences between the corresponding dose and vehicle were (2, 8), (7, 12) and (10, 17) (Table 2).

VCF_{max} was significantly increased 10 min after the three highest doses (Table 2). The maximal increase in this parameter were usually seen 20 min after the infusion; the percent increases at that time were 42, 39 and 77 % after the doses of 2, 3 and 5 mg, respectively (Fig. 2 A and 2 B).

The pattern of mean MRR was less consistent and the dose-effect relationship was less apparent than for other echocardiographic indices. Mean MMR 10 min after infusion was 127 mm · s^{-1} for vehicle, 144 mm · s^{-1} for 2 mg and 164 mm · s^{-1} for 5 mg. The difference after 5 mg compared to the vehicle was significant (95 % CI 7–58; Table 2).

Mean calculated CO values 10 min after infusion were 4.0 l · min^{-1} for vehicle, 4.3 l · min^{-1} for 1 mg, 5.2 l · min^{-1} for 2 mg and 6.2 l · min^{-1} for 5 mg. CO was significantly higher than after vehicle for 2, 3 and 5 mg doses (Table 2).

Mean calculated TPR was decreased by –464 (–701, –226), –479 (–738, –220) and –649 dyn · s · cm^{-5} after 2, 3, and 5 mg, respectively (Table 2).

Time-comparison analysis

The increase of 4 beats · min^{-1} in HR 0 and 5 min after infusion of 1 mg reached statistical significance. The mean increase after 2 mg was statistically significant and was similar to 1 mg at 5, 20, 30 and 60 min after infusion. After 5 mg a significant increase in HR was seen from 0 to 60 min after infusion. The maximum increase was 17 beats · min^{-1}, 60 min after infusion.

Increases in FS were significant from 10 to 30 min after infusion of 0.5 mg, from 0 to 30 min after infusion of 1 mg and from 0 to 60 min after infusions of 2, 3 and 5 mg (Fig. 1). VCF_{max} behaved similarly (Fig. 2 B).

Systolic time intervals

Mean QS2i 10 min after infusion was 528 ms after vehicle, 518 ms after 1 mg, 512 ms with 2 mg and 491 ms after 5 mg levosimendan (Table 2). The corresponding 95 % confidence intervals of the dose-effects after 1, 2 and 5 mg and vehicle were (–22, 2), (–28, –4) and (–50, –22).

Mean PEP and the PEP/LVET ratio were significantly shorter only after the 5 mg dose of levosimendan. Mean LVETi did not show any dose-effect response.

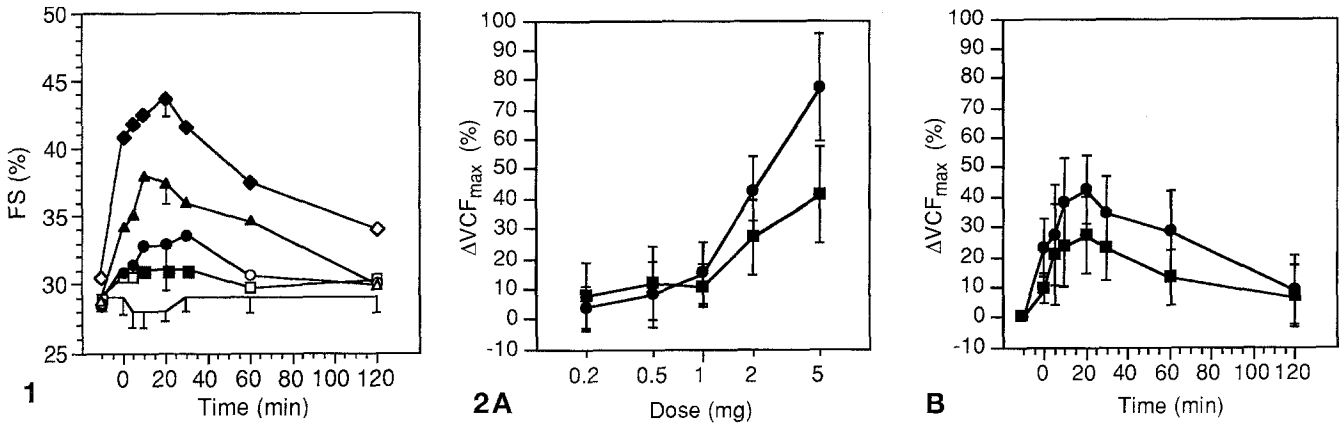

Fig. 1 Change in fractional shortening (FS, %) after 5-min infusions of vehicle (– $n = 8$) and 0.5 (□ $n = 8$), 1 (○ $n = 8$), 2 (△ $n = 8$) and 5 mg (◇ $n = 5$) of levosimendan in healthy volunteers. *Filled symbols* indicate significant changes ($P < 0.05$) compared to baseline (–10 min). Together with SEM values are shown changes 20 min after levosimendan and all changes after vehicle

Fig. 2 **A** Percent age changes (with 95 % CI) in maximal velocity of left ventricular circumferential fibre shortening (ΔVCF_{max}) 20 min after intravenous infusion of 0.2, 0.5, 1, 2 and 5 mg simendan (■) and levosimendan (●) in healthy subjects. ($n = 8$ for 0.2, 0.5 and 1 mg, and $n = 7$ for 2 and 5 mg of simendan; $n = 8$ for 0.2, 0.5, 1 and 2 mg and $n = 5$ for 5 mg of levosimendan). **B** ΔVCF_{max} (95 % CI) after intravenous infusion of 2 mg simendan and 2 mg levosimendan in healthy subjects ($n = 7$ for simendan and $n = 8$ for levosimendan)

Urine volume

The first urine fraction collected up to 2 h after infusion tended to be increased dose-dependently by 97 (–115, 310), 229 (8, 450) and 126 ml (–119, 371) after the doses of 2, 3 and 5 mg, respectively. Data from only 4 subjects were eligible for statistical analysis.

ECG

Mean PQ and mean QRS intervals were not affected by the levosimendan infusion. In comparison with the value 10 min after infusion of vehicle, the mean QT_c-interval was significantly increased by 34 (13, 56), 35 (13, 57) and 53 ms (28, 78) after the doses of 2, 3 and 5 mg. When mean QTc-intervals 10 min after levosimendan infusion were compared to values 10 min before infusion at each dose level, there were no statistically significant changes because the baseline value before the infusion was higher at higher doses than at lower doses.

Ambulatory ECG

Only a few isolated ventricular extrasystoles (VE) were noted. The highest individual hourly mean was 11.8 VE · h^{-1}. The subject concerned exhibited the highest VE rate in the study. There was no significant change in numbers of ventricular or supraventricular extrasystoles between the period before drug infusion, the period from the beginning of the infusion to 2 h after it and the period from 2 to 8 h after infusion. There was also no effect of dose on the frequency of ectopic beats.

Safety laboratory parameters

There were no clinically significant changes in any of the laboratory safety parameters. Some values lay outside the reference range, as often before as after the infusion. Mean serum potassium concentrations in the afternoon after the infusions were increased by 0.27 (0, 0.54), 0.27 (–0.02, 0.56), 0.28 (0.04, 0.52), 0.28 (0.08, 0.48), 0.22 (0.08, 0.36), 0.03 (–0.09, 0.15) and 0.22 (–0.11, 0.55) mmol · l^{1} compared to the morning values before the doses of 0, 0.2, 0.5, 1, 2, 3 and 5 mg, respectively.

Adverse events

There were no clinically significant or serious adverse events during the study. A few subjects reported smarting of the cannulated vein during the drug infusion. One subject experienced vertigo lasting for a few minutes, but with no change in blood pressure or heart rate. The vertigo occurred over 2 h after infusion of 2 and 3 mg, but not after the infusion of 5 mg.

Discussion

Noninvasive methods are preferable if healthy subjects are being studied. M-mode echocardiography and systolic time intervals are suitable methods if rapid alterations in the dimensions and contractility of left ventricle are to be evaluated. In our study, special attention was paid to obtaining good two-dimensional and M-mode echocardiographic image. In healthy subjects, volumes calculated from echocardiographically derived dimensions have been shown to be accurately correlated with volumes assessed using invasive methods [16].

The reliability of results can be estimated by measuring the repeatability, blindly if possible. In the present study the repeatability of digitisation was blindly measured in random order, but within-day repeatability was not blindly measured. This impaired the assessment of repeatability. The repeatability was good or satisfactory in relation to all variables measured and was comparable to that of other studies conducted using similar methods [22, 23].

In all measured haemodynamic parameters, there was a linear dose response. The effects of the 2 and 3 mg doses did not significantly differ from each other. In this study, for safety reasons the 3 mg dose was chosen for administration before the highest 5 mg dose. In a comparable dose-response study with racemic simendan there were quite marked haemodynamic effects after the two highest doses of 5 and 10 mg [12].

The heart rate remained stable and low throughout the study, up to the 2 mg dose. There was a marked increase in heart rate after the highest dose, which could be explained as a reflex reaction to peripheral vasodilatation. Heart rate may be regarded as not having any effect on the echocardiographic parameters measured [24, 25].

The diastolic blood pressure was decreased after the 0.5 mg dose, and there were substantial falls after the two highest doses. The pulse pressure increased even more, because the systolic blood pressure was also increased, although the increase in the latter was not significant. The changes in blood pressure suggest that the drug has a vasodilator effect, which would be in accordance with the findings in preclinical studies.

The most favourable haemodynamic profile was seen after 2 mg. In comparison with the baseline values, the calculated cardiac output was increased by 30 %, from 4.0 to 5.2 $l \cdot min^{-1}$, and the heart rate was increased by only 6 %, from 53 to 56 beats $\cdot$ min^{-1}. The increase in cardiac output may be regarded as the result of an increase in stroke volume by 28 %, from 75 to 96 ml. After a 5 mg dose there was a 72 % increase in cardiac output, resulting almost equally from increases in heart rate and stroke volume, of 26 and 33 % respectively.

Ejection fraction and fractional shortening are known to depend on loading conditions [25, 26]. The observed increases in both parameters could be explained by the change in loading conditions and the increased cardiac contractility. The increases in fractional shortening and ejection fraction could have been caused by decreases both in end-systolic diameter and volume, because they were dose-dependently decreased (Table 2).

Of the parameters measured here, heart-rate-corrected electromechanical systole is the most sensitive index of inotropy [27, 28], and it has been shown to be virtually independent of preload and afterload [17, 27, 29]. The significant dose-dependent decrease in this variable suggests a true increase in myocardial contractility not secondary to change in loading conditions (Table 2).

Relaxation of the myocardium is difficult to measure and interpretation of the results is still under debate [30]. In the present study the maximal relaxation values were the most variable of all the echocardiographic measurements. Heart rate and loading conditions are known to affect maximal relaxation [31]. Having regard to these factors, it was concluded that there was no indication of deterioration of myocardial relaxation but rather a tendency to an enhanced rate of relaxation.

In the ECG analyses a slight but statistically significant prolongation of the QT_c interval after doses of 1–5 mg levosimendan was noted in comparison with QT_c intervals 10 min after infusion of vehicle. The maximum increase in mean QT_c interval was 49 ms after the 5 mg dose, in comparison with the vehicle infusion, but only 21 ms in comparison with the value before the infusion. There was no significant prolongation of QT_c interval in comparison with baseline values at any dose level.

The implication of QT_c interval prolongation with regard to arrhythmogenecity of a drug is far from settled [32, 33]. On the basis of the findings in the present study, it was concluded that the minor prolongations in QT_c interval were of no clinical significance. However, data from larger number of treated subjects and especially from patients are required to assess the importance of this finding.

No proarrhythmic activity could be detected in ambulatory ECG recordings. Numbers of ventricular extrasystoles were very low and no dose-dependence was evident. The highest individual frequency of isolated ventricular extrasystole was 17 beats $\cdot$ h^{-1}, 5 h after a 3 mg dose. This is within normal limits in healthy subjects [34].

The only significant change in the laboratory safety parameters was a slight increase in serum potassium after the infusions. Because serum potassium tended to increase even after infusion of vehicle, and because there was no dose-dependence, it was concluded that infusion of the drug had not affected serum potassium, and the increase was apparently caused by other factors.

No sensation of discomfort was reported during or after infusion of the drug. There were no serious or unexpected adverse events. Smarting at the injection site was probably caused by the high concentration of the drug in the solutions infused. Whether there was a connection between the instances of sensations of vertigo and infusion of drug remains to be settled.

The calcium-sensitising effect and inhibition of PDE by other calcium-sensitisers, such as pimobendan and EMD 53998, is dueto to a particular enantiomer of each drug [35, 36]. In a previous study in a similar group and using a similar study design echocardiographic assessment but not systolic time intervals were used to explore the haemodynamic effects of simendan [12]. The effects of simendan and levosimendan on the heart rate were similar. After infusion of 5 mg of the drugs, heart rate increased by 7 beats $\cdot$ min^{-1} (0,14) after simendan and by 12 beats $\cdot$ min^{-1} (4,20) after levosimendan.

The dose-response of the mean maximal velocity of left ventricular circumferential fibre shortening 20 min after infusion is shown in Fig. 2 A. Up to 1 mg there was no difference between the compounds. After 2 and 5 mg the effects of levosimendan were about twice those of simendan. The effect of 2 mg levosimendan was similar to that of 5 mg simendan. This is also evident in studying the effects with time of 2 mg simendan and levosimendan (Fig. 2 B). Whether there were differences in the inotropic and vasodilator properties of the two compounds is impossible to say using the methods employed in these studies. The differences in haemodynamic parameters is in accordance with the preclinical results indicating that levosimendan is the active enantiomer of simendan.

Conclusions

Levosimendan enhanced left ventricular function in a dose-dependent manner in young healthy volunteers, due both to positive inotropic and vasodilator actions. Although there was a marked decrease in diastolic blood pressure, the effect on heart rate were moderate. Levosimendan was well tolerated.

References

1. Kass RS, Lederer WJ, Tsien RW, Weingart R (1978) Role of calcium ions in transient inward currents and aftercontractions induced by strophanthidin in cardiac Purkinje fibres. J Physiol 291: 187–208
2. Hasenfuss G, Holubarsch C, Just H, Blanchard E, Mulieri LA, Alpert NR (1987) Energetic aspects of iontropic interventions in rat myocardium. Basic Res Cardiol 82: 251–259
3. Beier N, Harting J, Jonas R, Klockow M, Lues I, Haeusler G (1991) The novel cardiotonic agent EMD 53998 is a potent calcium sensitizer. J Cardiovasc Pharmacol 18: 17–27
4. Ovaska M, Haikala H, Lundström K, Korkolainen T, Taskinen J, Lindén I-B (1992) Studies on calcium-dependent binding of simendan to troponin complex and troponin C. J Mol Cell Cardiol 24 [Suppl I]: S260
5. Haikala H, Lindén I-B, Pohto P (1992) Simendan, a novel calcium sensitizer, is as potent and efficient in the skinned fibre as in the electrically paced papillary muscle. J Mol Cell Cardiol 24 [Suppl I]: S260
6. Haikala H, Lindén I-B, Pohto P (1992) Changes in contraction-relaxation cycle by levosimendan indicate calcium sensitization in paced papillary muscle without impairment of relaxation (abstract) Eur Heart J 13 [Supol]: P765
7. Edes I, Papp J, Csanady M (1992) A unique effect of the new positive drug simendan (OR-1259) on the phosphorylation of key intracellular proteins (abstract). Eur Heart J 13 [Suppl]: 167
8. Edes I, Csanady M, Papp J (1993) The effects of new calcium sensitizers (levosimendan, pimobendan and EMD 53998) on myocardial protein phosphorylation (abstract) Eur Heart J 14 [Suppl]: 198
9. Edes I, Papp J, Csanady M (1991) The effects of a new calcium sensitizer, simendan, on intracellular protein phosphorylation (abstract). J Mol Cell Cardiol 24 [Suppl V]: S93
10. Raasmaja A, Talo A, Nissinen E, Lindén I-B, Pohto P (1991) Biochemical mechanisms of the novel cardiotonic agent OR-1259. J Mol Cell Cardiol 23 [Suppl V]: S129
11. Vegh A, Udvary E, Papp J (1992) Effects of simendan in a model of acute heart failure produced by severe regional ischaemia in dogs. J Mol Cell Cardiol 24 [Suppl V]: S45
12. Lilleberg J, Leikola-Pelho T, Sundberg S, Nieminen MS (1994) Hemodynamic effects of the novel cardiotonic drug simendan: echocardiographic assessment in healthy volunteers. Cardiovasc Drugs Ther 8: 263–269
13. Bazett HC (1920) An analysis of the time-relations of electrocardiogram. Heart 7: 353–370
14. Upton MT, Gibson DG (1978) The study of left ventricular function from digitized echocardiograms. Prog Cardiovasc Dis 20: 359–384
15. Roelandt J, Gibson DG (1980) Recommendations for standardization of measurements from digitized echocardiograms. Eur Heart J 1: 375–378
16. Teichholz LE, Kreulen T, Herman MV, Gorlin R (1976) Problems in echocardiographic volume determinations: echocardiographic-angiographic correlations in the presence or absence of asynergy. Am J Cardiol 37: 7–11
17. Li Q, Belz GG (1993) Systolic time intervals in clinical pharmacology. Eur J Clin Pharmacol 44: 415–421
18. Weissler AM, Harris WS, Schoenfeld CD (1968) Systolic time intervals in heart failure in man. Circulation 37: 149–159
19. Spodick DH, Doi YL, Bishop RL, Hashimoto T (1984) Systolic time intervals reconsidered. Reevaluation of the preejection period: absence of relation to heart rate. Am J Cardiol 53: 1667–1670
20. Sundberg S (1986) Influence of heart rate on systolic time intervals. Am J Cardiol 58: 1144–1145
21. Bland JM, Altman DG (1986) Statistical methods for assessing agreement between two methods of clinical measurement. Lancet I: 307–310
22. Kupari M (1984) Reproducibility of M-mode echocardiographic assessment of left ventricular function. Significance of the temporal range of measurements. Eur Heart J 5: 412–418
23. Groundstroem K, Huikuri H, Korhonen U, Ikäheimo M, Heikkila J, Takkunen J (1987) Comparison of echocardiographic and radionuclide methods with contrast angiography assessment of left ventricular function – response to isometric exercise in subjects without definite heart disease. Ann Clin Res 19: 187–193
24. DeMaria AN, Neumann A, Schubart PJ, Lee G, Mason DT (1979) Systematic correlation of cardiac chamber size and ventricular performance determined with echocardiography and alterations in heart rate in normal persons. Am J Cardiol 43: 1–9
25. Quinones AM, Gaasch WH, Cole JS, Alexander JK (1975) Echocardiographic determination of left ventricular stress-velocity relations in man with reference to the effects of loading and contractility. Circulation 51: 689–700
26. Quinones MA, Gaasch WH, Alexander JK (1976) Influence of acute changes in preload, afterload, contractile state and heart rate on ejection and isovolumic indices of myocardial contractility in man. Circulation 53: 293–302
27. Gibson DG (1978) Use of the systolic time intervals in clinical pharmacology. Br J Clin Pharmacol 6: 97–102
28. Belz GG, Stern HC, Butzer R (1985) Dose-response following single administrations of a new cardiac performance enhancer RO 13-6438 in normal volunteers. J Cardiovasc Pharmacol 7: 86–90
29. Stern HC, Matthews JH, Belz GG (1984) Influence of dihydralazine induced afterload reduction on systolic time intervals and echocardiography in healthy subjects. Br Heart J 52: 435–439
30. Plotnick GD (1989) Changes in diastolic function – difficult to measure, harder to interpret. Am Heart J 118: 637–641
31. Bahler RC, Vrobel TR, Martin P (1983) The relation of heart rate and shortening fraction to echocardiographic indexes of left ventricular relaxation in normal subjects. J Am Coll Cardiol 2: 926–933

32. Sagie A, Larson MG, Goldberg RJ, Bengtson JR, Levy D (1992) An improved method for adjusting the QT interval for heart rate (the Framingham Heart Study). Am J Cardiol 70: 797–801
33. Morganroth J, Brozovich FV, McDonald JT, Jacobs RA (1991) Variability of the QT measurement in healthy men, with implications for selection of an abnormal QT value to predict drug toxicity and proarrhythmia. Am J Cardiol 67: 774–776
34. Viitasalo MT, Kala R, Eisalo A (1982) Ambulatory electrocardiographic recording in endurance athletes. Br Heart J 47: 213–220
35. Lues I, Beier N, Jonas R, Klockow M, Haeusler G (1993) The two mechanisms of action of racemic cardiotonic EMD 53998, calcium sensitization and phosphodiesterase inhibition, reside in different enantiomers. J Cardiovasc Pharmacol 21: 883–892
36. Endoh M, Shibasaki T, Satoh H, Norota I, Ishihata A (1991) Different mechanisms involved in the positive inotropic effects of benzimidazole derivative UD-CG 115 BS (pimobendan) and its demethylated metabolite UD-CG 212 Cl in canine ventricular myocardium. J Cardiovasc Pharmacol 17: 365–375