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Pharmacokinetics of levosimendan and its circulating metabolites in patients with heart failure after an extended continuous infusion of levosimendan

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Aims
The purpose of the study was to characterize the pharmacokinetics of levosimendan and its metabolites OR-1855 and OR-1896 in patients with congestive heart failure.

Methods
Levosimendan was administered as a continuous intravenous infusion for 7 days. Twelve subjects received the drug at an infusion rate of 0.05 μg kg^{-1} min^{-1} and 12 at a rate 0.1 μg kg^{-1} min^{-1}.

Results
Steady state concentrations of levosimendan were achieved within 4 h. Peak concentrations of the metabolites occurred after termination of the infusion. The mean (± SD) half-life of the active metabolite OR-1896 was 81 ± 37 h after the lower dose and 81 ± 28 h after the higher dose ($P = 0.992$, 95% confidence interval on the difference −27.5, 27.7).

Conclusions
The metabolites of levosimendan, OR-1855 and OR-1896, were formed and eliminated slowly, their peak concentrations occurring after termination of the 7-day infusion of the drug.

Introduction

Levosimendan is a new calcium sensitizer intended for the treatment of congestive heart failure. The pharmacokinetics of levosimendan have been well described [1, 2], but those of its circulating metabolites OR-1855 and OR-1896 have not been reported in detail. In a previous study it was noted that the concentrations of the metabolites were still increasing 30 h after termination of a 24 h infusion of the drug [3]. Since the acetylated metabolite OR-1896 has been shown to have similar pharmacological properties as levosimendan [4], we have studied the pharmacokinetics of both levosimendan and its metabolites in patients with congestive heart failure. It was assumed that a 7-day infusion period

would be needed to obtain steady state concentrations of the metabolites and a 10–15 day follow-up period was chosen to characterize the elimination of the metabolites more thoroughly. The binding of the metabolites to plasma protein was also studied.

Methods

Study design and subjects

An open, non-randomized phase II study was performed in two study centres. Twelve patients received levosimendan at a continuous intravenous infusion rate of 0.05 μg kg^{-1} min^{-1} for 7 days and 12 others at a rate of 0.1 μg kg^{-1} min^{-1} for 7 days. The follow-up period was 10 days for six patients receiving the dose of 0.1 μg kg^{-1} min^{-1} and 13–15 days for the rest of the patients [5]. Renal function of the subjects was assessed by determining creatinine clearance at baseline. It was classified as follows: normal renal function CL_{cr} >80 ml min^{-1} 1.73 m^{-2}, mild impairment CL_{cr} 50–80 ml min^{-1} 1.73 m^{-2}, moderate CL_{cr} 30–49 ml min^{-1} 1.73 m^{-2} and severe impairment CL_{cr} <30 ml min^{-1} 1.73 m^{-2}. Renal function was normal in 14 patients, mildly impaired in five patients, moderately impaired in three patients and severely impaired in two patients.

The study protocol and the informed consent form were approved by the Ethics Committees of the study hospitals (Helsinki University Central Hospital, Helsinki, Finland and Mustamäe Hospital, Tallinn, Estonia). Written informed consent was obtained from all patients. The study followed the recommendations for biomedical research involving humans found in the current version of the Declaration of Helsinki.

Blood sampling

Blood samples (3 ml) for the determination of plasma concentrations of levosimendan and its metabolites OR-1855 and OR-1896 were drawn before drug administration (0 h) and 2, 4, 8, and 12 h after starting the infusion. Thereafter, blood samples were drawn daily in the morning of study days 2 through 7 during the infusion. After stopping the infusion on study day 8, samples were drawn at 0 min and at 0.5, 1, 2, 4, 12, 24 and 36 h post-infusion. Thereafter, samples were drawn daily at 09.00 h (every 24 h) until 10–15 days post-infusion. Blood was centrifuged within 10 min of sampling. The plasma was separated and transferred into two polypropylene tubes, frozen immediately and kept at −70 °C until analysis.

The *ex vivo* protein binding of levosimendan, OR-1855 and OR-1896 was determined by ultracentrifugation at 1500 *g* for 10 min, followed by analysis of the supernatant fraction.

All samples were analysed using a validated high performance liquid chromatographic/tandem mass spectrometric (LC-MS/MS) method. The sample preparation involved addition of internal standards and liquid-liquid extraction with a mixture of ethyl acetate and hexane. The LC system consisted of a HP Model 1090 Series II pump and an autosampler (Hewlett Packard, USA). All separations were performed with LiChrosorb RP-18 250 × 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 instrument was operated in the multiple reaction monitoring (MRM) mode.

For levosimendan, the intra-assay precision (% CV) ranged from 0.4% to 11% in plasma and from 0.7% to 2.9% in supernatant. For OR-1855, the intra-assay precision ranged from 1.8% to 13% in plasma and from 1.4% to 12% in supernatant. The intra-assay precision for OR-1896 ranged from 1.1% to 10% in plasma and from 2.9% to 5.2% in supernatant.

The interassay precision and accuracy were determined from the analysis of quality control samples. The interassay 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 target.

The limit of quantification was 0.2 ng ml^{-1} for levosimendan in each of the matrices. For OR-1855 the limit of quantification was 1.0 ng ml^{-1} in plasma and 0.5 ng ml^{-1} in supernatant. For OR-1896 the limit of quantification was 0.2 ng ml^{-1} in plasma and in supernatant.

Pharmacokinetics

Since there was a limited number of sampling points during the distribution phase, the pharmacokinetic parameters of levosimendan and its metabolites OR-1855 and OR-1896 were determined using noncompartmental methods. Parameters were obtained using the WinNonlin Professional computer program (Version 1.5, Pharsight Corporation, Mountain View, California).

Protein binding

The percentage of free unbound fraction (*f*u) of levosimendan, OR-1855 or OR-1896 was calculated as follows from the equation:

$$fu(\%) = (C_{supernatant}/C_{plasma}) \times 100\%$$

where *C* is the concentration in supernatant or in plasma.

Statistical analysis

Variables were compared between the two infusion rates using an ANOVA with an effect for the infusion rate. Pearson's and Spearman's correlation coefficients between pharmacokinetic variables were calculated.

Results

All patients except two were male. Three patients in the higher and one in the lower levosimendan dose group had New York Heart Association Class (NYHA) IV heart failure. All other patients were in NYHA class III.

Steady state concentrations of levosimendan were achieved within 4 h. There were no statistically significant differences between the two doses with respect to half-life, clearance and volume of distribution (Table 1). A dose proportional increase in AUC and C_{ss} was seen between the doses ($P < 0.0001$).

The concentration of the metabolites OR-1855 and OR-1896 tended to increase after discontinuation of levosimendan. The increase in C_{max} was dose-proportional. The half-life values of the metabolites were considerably longer compared with that observed for the parent drug (Table 2). There were no statistically significant differences in half-lives of the metabolites between the two doses.

The plasma protein binding of levosimendan and its metabolites was studied only in patients administered the lower dose of levosimendan (0.05 μg kg^{-1} min^{-1}). The mean free fraction of levosimendan was 3.0% (range 1.9–5.4%). The mean free plasma fractions of the metabolites were considerably higher, with a mean (range) for OR-1855 of 58% (51–67%) and for OR-1896 61% (52–67%).

The clearance of levosimendan did not correlate with creatinine clearance ($P = 0.259$). Neither were there any correlations between the dose-normalized AUC values for OR-1855 nor OR-1896, and creatinine clearance ($P = 0.9137$ and 0.8913, respectively).

Discussion

The pharmacokinetics of levosimendan after a continuous infusion for 7 days were found to be similar to those observed previously in single dose studies. The parameters calculated by non-compartmental methods in the present work were also similar to those estimated by two-compartmental modelling methods [1, 2]. Steady-state concentrations for levosimendan were reached within 4 h and remained constant throughout the infusion. There was a dose-proportional relationship in C_{ss} and AUC between the doses, indicating that

Table 1
Pharmacokinetic parameters for levosimendan calculated by noncompartmental methods after a 7 day intravenous infusion of the drug (mean ± SD)

	Levosimendan		
	0.05 μg kg^{-1} min^{-1} ($n = 12$)	0.1 μg kg^{-1} min^{-1} ($n = 12$)	*P* value (95% confidence intervals on the differences)
C_{ss} (ng ml^{-1})	14.9 ± 3.6	34.6 ± 8.5	$P < 0.0001$ (–25.2, –14.2)
AUC(0,∞) (ng ml^{-1} h)	2510 ± 620	5970 ± 1480	$P < 0.0001$ (–4419, –2497)
CL_{tot} (l h^{-1} kg^{-1})	0.21 ± 0.05	0.18 ± 0.05	0.129 (–0.011, 0.077)
CL_{tot} (l h^{-1})	17.9 ± 6.4	14.4 ± 1.4	0.140 (–1.26, 8.39)
V_z (l kg^{-1})	0.33 ± 0.12	0.34 ± 0.18	0.828 (–0.14, 0.11)
V_z (l)	27.8 ± 11.5	28.2 ± 16.7	0.955 (–12.5, 11.8)
$t_{1/2}$ (h)	1.1 ± 0.2	1.4 ± 0.7	0.160 (–0.76, 0.13)

C_{ss} steady state concentration, AUC(0,∞) area under the curve to infinity, CL_{tot} total plasma clearance, V_z volume of distribution, $t_{1/2}$ half-life.

Table 2
Pharmacokinetic parameters for OR-1855 and OR-1896 after an intravenous infusion of levosimendan for 7 days (mean ± SD)

	OR-1855			OR-1896		
	0.05 μg kg^{-1} min^{-1} (n = 12)	0.1 μg kg^{-1} min^{-1} (n = 12)	P value (95% CI)	0.05 μg kg^{-1} min^{-1} (n = 12)	0.1 μg kg^{-1} min^{-1} (n = 12)	P value (95% CI)
C_{max} (ng ml^{-1})	7.8 ± 5.1	18.1 ± 11.2	0.009 (–17.8, –2.9)	9.9 ± 4.7	17.1 ± 9.8	0.030 (–13.7, –0.76)
t_{max} (h)*	168 (144–314)	170 (144–216)	0.301 (–23.4, 57.6)	180 (144–264)	170 (144–264)	0.873 (–24.4, 28.6)
AUC(0,last) (ng ml^{-1} h)	1570 ± 1220	3560 ± 2130	0.011 (–3477, –501)	2120 ± 1260	3450 ± 2050	0.070 (–2770, 118.3)
AUC(0,∞) (ng ml^{-1} h)	1650 ± 1260	3950 ± 2420	0.015 (–4085, –505)	2370 ± 1820	3890 ± 2540	0.107 (–3388, 356.8)
$t_{1/2}$ (h)	72.6 ± 17.8	78.4 ± 27.8	0.579 (–27.4, 15.8)	81.3 ± 37.1	81.2 ± 27.5	0.992 (–27.5, 27.7)

**median (range). C_{max} peak concentration, t_{max} time to peak concentration, AUC(0,last) area under the curve to the last measured concentration, AUC(0,∞) area under the curve to infinity, $t_{1/2}$ half-life, 95% CI 95% confidence intervals on the differences.*

pharmacokinetics of levosimendan are linear over this dose range.

The duration of action of levosimendan is much longer than expected on the basis of the half-life of the parent drug. The active metabolite OR-1896 is formed slowly and may explain partly the long-lasting haemodynamic effects seen with levosimendan [3, 6]. This metabolite is also bound less to plasma proteins making its effects greater [1].

Ten patients included in this study had mild to severe renal dysfunction. However, the present results suggest that impaired renal function does not affect the pharmacokinetics of levosimendan or its metabolites, although a relatively small number of patients were studied. It is possible that the improved haemodynamics caused by levosimendan [7] may have enhanced the renal function, thereby masking any potential effects of the disease on pharmacokinetics.

In conclusion, peak concentrations of levosimendan were achieved within 4 h of the start of a continuous intravenous infusion. Since the half-lives of the circulating metabolites were about 80 h, steady state concentrations were not reached during the 7-day infusion, their concentrations increasing after its termination.

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