Oral Levosimendan Increases Cerebral Blood Flow Velocities in Patients with a History of Stroke or Transient Ischemic Attack: A Pilot Safety Study

Matti Kivikko, MD, PhD[1,2],*, Mikko Kuoppamäki, MD, PhD[2], Lauri Soinne, MD, PhD[3], Stig Sundberg, PhD[4], Pasi Pohjanjousi, MSc[2], Juha Ellmen, PhD[2], Risto O. Roine, MD, PhD[5]

[1] Department of Cardiology, Helsinki University Central Hospital, Helsinki, Finland
[2] Orion Pharma, Espoo, Finland
[3] Department of Neurology, Helsinki University Central Hospital, Helsinki, Finland
[4] Department of Biosciences, Division of Physiology and Neurosciences, University of Helsinki, Helsinki Finland
[5] Division of Clinical Neurosciences, Turku University Hospital, Turku, Finland

A R T I C L E   I N F O

A B S T R A C T

Background: Intravenous levosimendan is indicated for acute heart failure. The compound has shown promising beneficial effects in ischemic stroke models.

Objective: We evaluated the efficacy and safety of oral levosimendan in patients with a history of cerebral ischemia.

Methods: In a randomized, double-blind, placebo-controlled, parallel-group study, 16 patients with a history of ischemic stroke/transient ischemic attack received oral levosimendan in 5 escalating doses from 0.125 to 2.0 mg daily for 18-day intervals of each dose; 5 patients received placebo. Twenty-four-hour ambulatory ECG and cerebral blood flow velocities using transcranial Doppler ultrasound were recorded at baseline and at the end of each dosing period. Vasomotor reactivity was assessed via the breath holding index. In addition, plasma levels of N-terminal-pro-B-type natriuretic peptide (NT-pro-BNP) and the metabolites of levosimendan were determined.

Results: Levosimendan induced an increase in cerebral blood flow velocities and a decrease in NT-pro-BNP compared with placebo. There was no significant effect on breath holding index. Doses ≥ 0.5 mg increased heart rate by 5 to 9 beats/min. The dose level of 2.0 mg exceeded the preset safety margin of ventricular extrasystoles per hour (ie, upper 90% CI of the ratio of levosimendan to placebo above 2) with an estimate of 3.10 (90% CI, 0.95–10.07).

Conclusions: Oral levosimendan increases cerebral blood flow velocities and diminishes NT-pro-BNP levels in patients with earlier ischemic cerebrovascular event. Daily doses up to 1.0 mg were well tolerated, whereas the 2.0 mg dose level induced an increase in ventricular extrasystoles. ClinicalTrials. gov identifier: NCT00698763.

Introduction

Levosimendan is a calcium sensitizer with vasodilatory and cardioprotective properties[1] and is used as an intravenous treatment for acute heart failure. It has been shown to exert beneficial hemodynamic and neurohormonal effects as well as reduce symptoms in this patient population.[2–5]

Promising preclinical findings with levosimendan have recently shifted the research interest into a new therapeutic area: Prevention of ischemic stroke. Orally administered levosimendan has been shown to significantly improve survival in preclinical models of primary and secondary prevention of stroke. These studies also suggested a beneficial synergism in coadministration of levosimendan with angiotensin II receptor blockers (ARBs).[6] In a model of transient brain ischemia by intraluminal occlusion of the middle cerebral artery in 40 male Wistar rats, intravenously administered levosimendan limited the infarct size and brain swelling by 40% and 53%, respectively, but no effect on neurologic outcome or mortality could be demonstrated.[7]

The putative mechanisms underlying the beneficial effects of levosimendan may be related to its vasodilatory and antiischemic properties,[1,8] improved endothelial function,[9] and antiaggregatory effect on platelets.[10]

* Address correspondence to: Matti Kivikko, MD, PhD, Orion Pharma, Orionintie 1, PO Box 65, FI-02101 Espoo, Finland.
   E-mail address: matti.kivikko@orionpharma.com (M. Kivikko).

The clinical data on orally administered levosimendan are limited to patients with heart failure. The largest study with oral levosimendan, the PERSIST (effects of PERoral levoSImendan in the prevention of further hoSpTalisations in patients with chronic heart failure) study[11] was a placebo-controlled study of 307 patients with severe chronic heart failure. Levosimendan doses were 1 or 2 mg daily and the exposure was at least 180 days. Levosimendan improved the quality of life, decreased N-terminal-pro-B-type natriuretic peptide (NT-pro-BNP) levels, and improved renal function, but resulted in an increase in heart rate (HR) of about 8 beats/min. No significant differences in the occurrence of atrial or ventricular arrhythmias were seen.

We performed a pilot study for preliminary evaluation of potential benefits and safety of levosimendan in patients with an earlier ischemic cerebrovascular event. Because the eventual proarrhythmogenic effect of oral levosimendan is unsettled, we included a thorough assessment of arrhythmias in our study. Our primary objective was to explore the safety of oral levosimendan and the primary safety variable was the number of ventricular extrasystoles per hour (VES/h) in the 24-hour ambulatory ECG monitoring. The secondary objective was to evaluate the potential effect of levosimendan on cerebral circulation.

Methods

The study was conducted in accordance with the ethical principles of the Declaration of Helsinki-Good Clinical Practice Guideline, and the regulatory requirements. All patients provided written informed consent before performance of any study procedure.

Design

This was a multicenter, Phase II, randomized, double-blind, placebo-controlled, parallel-group study. The study was carried out in 9 academic neurology centers in Finland, Germany, Hungary, and Sweden. After randomization, all patients first received single-blind placebo for a maximum of 18 days (placebo run-in). During the double-blind phase, 5 escalating doses of oral levosimendan were given for a maximum of 18 days in 5 treatment periods. The doses of levosimendan were 0.125, 0.25, 0.5, 1.0, and 2.0 mg once daily. Patients randomized to placebo received placebo throughout all 5 periods.

Patients

Patients within the age range of 50 to 80 years with ischemic stroke or transient ischemic attack (TIA) within 1 to 9 months before the screening visit were included. Patients had to be receiving maintenance treatment with an ARB or angiotensin-converting enzyme inhibitor, cholesterol-lowering agent, and an antiaggregatory agent, started at least 1 month before the screening visit.

The main exclusion criteria were stroke or TIA due to cardiac embolism, vasculitis, or arterial dissection, a history of life-threatening ventricular arrhythmias, or ventricular tachycardia in the 24-hour ambulatory ECG at screening. Patients with severe hemiparesis or dysphasia inhibiting the ability to fully comply with the study protocol requirements were also excluded. In practice, the exclusion and inclusion criteria restricted the patient selection to a sample of stable patients with either a previous TIA or a minor stroke of small-vessel and/or large-vessel etiology.

Assessments

Cerebral blood flow velocities (peak, end diastolic, and mean) at rest and after a 30-second breath holding were assessed at baseline and at the end of each treatment period with transcranial Doppler (TCD) ultrasonography.[12] Each site used its own TCD equipment, but a manual for performing the procedure was created and presented at an investigators' meeting before the initiation of the study. The flow velocities were measured via transtemporal window, preferably from the side ipsilateral to the ischemic event. However, if the affected side was clearly more difficult to visualize, then the contralateral side was used. If the testing at screening visit could not be performed technically reliably, the patient was to be excluded from the study. In consecutive measurements during the study, the same side for an individual patient was used. The blood flow velocities were measured from the M1 segment level of the middle cerebral artery. Before each TCD examination, the patient rested for 5 to 10 minutes lying in a supine position with head straight. At the end of the resting period, blood pressure and HR were recorded.

Cerebrovascular reactivity to hypercapnia was assessed by means of breath holding index (BHI) using TCD ultrasonography. Mean blood flow velocity of the middle cerebral artery was measured at rest (Vrest) and at the end of a breath holding period of at least 30 seconds (VBH).

BHI was calculated using the following formula:

$$BHI = 100 \times [(VBH{-}Vrest)/Vrest]/T$$

where T is the period of breath holding in seconds, VBH is the mean blood flow velocity at the end of the breath holding period, and Vrest is the mean blood flow velocity at baseline during normal breathing.

A 24-hour ambulatory 3-lead ECG (Holter) was recorded at screening and at the end of each treatment period. The Holter recordings were analyzed by a single central laboratory, which also provided the recorders for the study centers. The central laboratory sent the analysis report to the study center, and the dose was not increased until the report was available. The stopping rules for dose escalation were a mean 24-hour HR increase of > 15 beats/min compared with screening and, at the same time, HR was to be > 90 beats/min or the patient had to have symptoms related to increased HR; ventricular tachycardia > 10 consecutive beats; symptomatic atrial fibrillation requiring intervention; serious adverse event; or the best interest of the patient as judged by the investigator.

Plasma samples for the determination of NT-pro-BNP and the levosimendan metabolites OR-1896 and OR-1855 were drawn at baseline and at the end of each dosing period. The samples were analyzed in central laboratories. A detailed description of the analysis method for the metabolites has been published.[13]

Study Monitoring

The study was monitored by the sponsor (Orion Pharma, Espoo, Finland). All study data were monitored and collected according to the protocol (and amendments) and recorded on the sponsor's study-specific electronic case report forms using electronic data capture. The study monitor verified that the case report forms corresponded with source data. For this purpose, the study monitor was allowed direct access to hospital or patient records and original laboratory data as far as they were related to the study.

Statistical Methods

The primary objective of our study was to explore the safety of different doses of oral levosimendan in patients with an earlier history of ischemic cerebrovascular event.

The number of VES/h in the 24-hour ambulatory ECG was the primary safety variable in the study. The Holter recording of the

placebo run-in period was used as the baseline reference for all changes. Number of VES/h, the cerebral blood flow data from TCD ultrasonography, vital signs, and NT-pro-BNP were summarized for each dose level by randomization group, using descriptive statistics. Repeated measures ANCOVA model with randomized treatment and center as between factors, dose level as within factor, and baseline value as covariate, were used to evaluate the differences between randomized treatment groups. Contrasts were used to evaluate the safety of each dose level; that is, each levosimendan dose was compared with placebo. In statistical analysis, log-transformed VES/h data were used to ensure normality.

The primary safety objective was to show that there is at least 1 safe dose level of oral levosimendan compared with placebo. A dose level doubling the prevalence of VES/h was considered potentially proarrhythmic and unsafe (ie, levosimedan:placebo > 2.00). Previous data suggest reasonable log-linear relationships between the dose of levosimendan and the number of VES/h.[14] Based on those assumptions, bootstrap simulation (5000 simulations) was used to evaluate probabilities of a dosing group not exceeding the safety margin (ie, levosimedan:placebo > 2.00). Evaluation of the safe dose was based on 2-sided 90% CIs; if the upper limit of 90% CI lies above 2.00, it was to be concluded that the dose is not safe. Simulations showed that 45 patients, randomized in 2:1 allocation, would provide > 90% power to show that the median dose (ie, 0.5 mg) is safe and to elicit a dose-dependent response.

Results

Due to the slower-than-anticipated recruitment, the study was prematurely discontinued. In total, 32 patients were screened, of whom 11 were excluded for not meeting the eligibility criteria (**Figure 1**). Thus, only 21 patients were included. Further, the preplanned randomization ratio of 2:1 to levosimendan and placebo turned out to be 3:1. Sixteen patients received
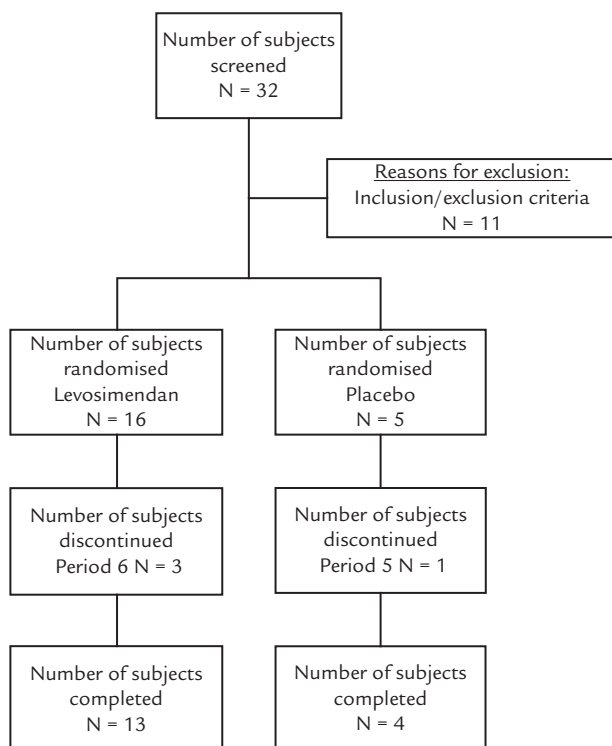
levosimendan and 5 received placebo on top of their concomitant medical treatment. Using the same log-linear relation as in the original sample size calculations with 2-sided 90% CIs, it was estimated that 20 study patients would still provide approximately 75% power to show that the median dose is safe.

Four out of the 21 patients (3 in the levosimendan group and 1 in the placebo group) did not receive all the dose levels. The placebo patient discontinued because a stopping rule was met (ventricular tachycardia of > 10 consecutive beats), whereas 1 levosimendan patient discontinued due to the occurrence of second-degree atrioventricular block, 1 levosimendan patient discontinued for adverse event (depression; best interest of a patient as judged by the investigator) and 1 levosimendan patient discontinued for serious adverse event (sepsis, apnea, or epilepsy). All discontinuations took place at the final dose level (ie, 2.0 mg daily). These discontinued patients were included in the intention-to-treat analysis.

Baseline and demographic characteristics were comparable between the treatment groups (**Table 1**).

Cerebral Blood Flow

Levosimendan increased cerebral blood flow velocities both at rest and after breath holding without any apparent dose effect. In **Figure 2**, the mean blood flow velocities are shown for individual dose levels both at rest and after breath holding. When the results of all levosimendan dose levels were pooled and compared with placebo, a statistically significant difference ($P < 0.001$) from placebo was seen, both at rest and at the end of the breath holding. At individual dose levels, the difference in the change from baseline was significant ($P < 0.05$) when compared with placebo at rest with the lower levosimendan doses (0.125 and 0.25 mg); doses ≥ 0.5 mg only achieved numerically increased blood flow velocities. At the end of the breath holding the pattern was similar, with the significant differences seen with the lower doses only.

In BHI, there was no difference between placebo and the pooled levosimendan dose levels - 0.77 [0.18] vs 0.76 [0.09]; $P = 0.977$ (values expressed as mean [SEM]). Also, at individual dose levels, no significant differences to placebo were observed.

Arrhythmias

To assess the safe dose levels of levosimendan, the baseline-adjusted number of VES/h at each dose level was compared with the corresponding values in the placebo group (**Figure 3**). Although the predefined safety limit (upper 90% CI in levosimendan:placebo > 2) was marginally exceeded with the dose of 0.5 mg (but not with 0.125, 0.25, and 1.0 mg), only the highest dose of 2.0 mg definitely exceeded the limit (levosimendan:placebo estimate 3.10; lower 90% CI, 0.95; upper 90% CI, 10.07).

When inspecting the data from individual patients, it is evident that the number of VES/h was fairly constant throughout the study period in all patients in the placebo group. Of the 16 patients in the levosimendan group, 10 (63%) had virtually no changes in the number of VES/h throughout the study. On the other hand, 4 patients (25%) showed a consistent pattern with increases in the number of VES/h at higher doses. At the 2 lowest doses (ie, 0.125 and 0.25 mg) none of the patients showed any appreciable increase in the number of VES/h.

No episodes of sustained ventricular tachycardia were recorded. Nonsustained ventricular tachycardia occurred in 5 levosimendan patients (31%) and in 2 placebo patients (40%). Furthermore, only 1 patient taking levosimendan seemed to have a distinct pattern with the occurrence of nonsustained ventricular tachycardia at all doses from 0.25 mg and above. In the remaining patients, the

Figure 1. Subject disposition. Period 5 refers to dose level 1 mg daily and period 6 to dose level 2 mg daily.

Table I
Summary of demographic and baseline characteristics.

Variable	Levosimendan (n = 16)	Placebo (n = 5)
Male gender[*]	8 (50)	3 (60)
Age, y[†]	65 (9)	64 (9)
Previous cerebrovascular ischaemic event (within 1–9 mo)[*]		
Ischemic stroke	12 (75)	2 (40)
Transient ischaemic attack	4 (25)	3 (60)
Hypertension[*]	12 (75)	4 (80)
Diabetes mellitus[*]	4 (25)	1 (20)
Body mass index[†]	27 (3)	27 (5)
Systolic blood pressure, mm Hg[†]	138 (15)	143 (16)
Diastolic blood pressure, mm Hg[†]	79 (10)	86 (12)
Heart rate, beats/min[†]	72 (11)	78 (10)
Ventricular extrasystoles per hour[†]	4.8 (5)	6.6 (10)
Selected concomitant medications[*]		
Angiotensin-converting enzyme inhibitors/ angiotensin II receptor blockers	16 (100)	5 (100)
Lipid-lowering agent	16 (100)	5 (100)
Aspirin alone or with dipyridamole	13 (81)	5 (100)
Dipyridamole alone or with aspirin	5 (31)	2 (40)
Clopidogrel	2 (13)	0 (0)
Transcranial Doppler variables[†]		
At rest		
Mean flow velocity, cm/sec	48 (15)	48 (13)
End diastolic flow velocity, cm/sec	35 (12)	37 (9)
Peak flow velocity, cm/sec	75 (22)	72 (21)
After 30-sec breath holding		
Mean flow velocity, cm/sec	58 (22)	64 (19)
End diastolic flow velocity, cm/sec	44 (19)	50 (14)
Peak flow velocity, cm/sec	83 (31)	91 (31)

[*] Values are given as n (%).
[†] Values are given as mean (SD).

ventricular tachycardias occurred only at intermediate doses. The maximum length of the nonsustained ventricular tachycardia was 10 beats in the levosimendan group and 16 beats in the placebo group.
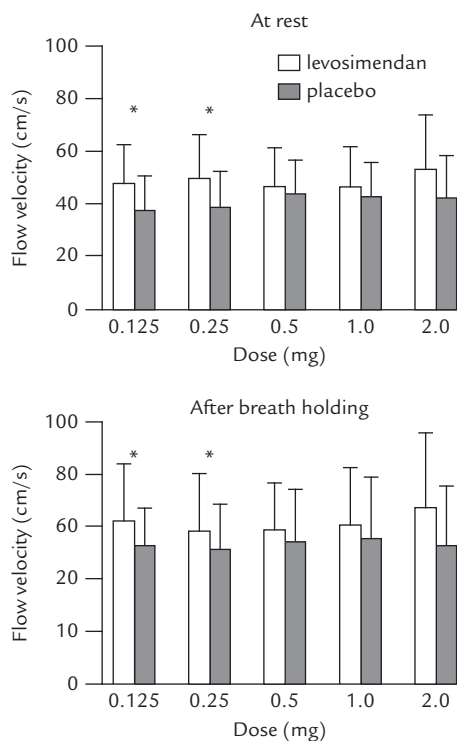

Figure 2. Mean (SD) blood flow velocities of the middle cerebral artery at different dose levels. (* < 0.05).

No episodes of atrial fibrillation or flutter were observed in either group. Supraventricular tachycardia occurred indiscriminately at some time point in the majority of patients in both treatment groups.

HR and Blood Pressure

In the levosimendan group, the 24-hour mean HR was 72 beats/min at baseline and remained constant with 0.125 and 0.25 mg doses. Thereafter, the mean HR increased to 77, 78, and 80 beats/min with 0.5, 1.0, and 2.0 mg doses, respectively. In the placebo group, the mean HR varied between 69 and 80 beats/min at different time points, without any definite trend. At doses of 0.5, 1.0, and 2.0 mg, the difference between levosimendan and placebo groups was statistically significant ($P = 0.017$, $P = 0.023$, and $P < 0.0001$, respectively).

There were no changes in systolic blood pressure with any of the levosimendan doses. Diastolic blood pressure decreased statistically significantly only with the highest levosimendan dose (ie, 2.0 mg), by 6 mm Hg ($P = 0.022$).

NT-Pro-BNP

The median NT-pro-BNP values at baseline were 129 ng/L and 127 ng/L in the levosimendan and placebo groups, respectively. In the levosimendan group, the median NT-pro-BNP values decreased from baseline during active treatment periods by 21% to 58% (**Figure 4**). The decrease was statistically significantly different from placebo with doses 0.125, 1.0, and 2.0 mg ($P = 0.039$, $P = 0.021$, and $P = 0.034$, respectively).

Pharmacokinetic Properties

The plasma levels of levosimendan metabolites OR-1855 and OR-1896 increased in a dose-dependent manner. Plasma concentrations at the end of each dosing level are shown in **Figure 5**. There were no significant correlations between the metabolite levels and the mean cerebral blood flow (data not shown).

Tolerability and Safety

The most common adverse event was ventricular tachycardia, which was reported in 3 levosimendan-treated patients (19%) and 1 placebo patient (20%). Sinus tachycardia, chest discomfort, gastroenteritis, back pain, and headache were reported in 2 patients receiving levosimendan each (13%). Sinus tachycardia was reported by 1 patient (20%) and headache by 1 patient (20%) in the placebo group.

Serious adverse events were reported in 2 patients receiving levosimendan treatment: 1 had VES at the 2.0 mg dose level and the other had sepsis, epilepsy, and apnea also at the 2.0 mg dose
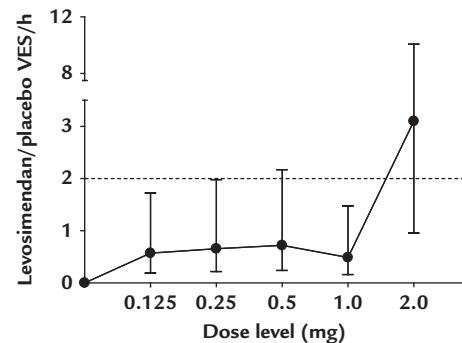

Figure 3. Statistical model based estimates (and 90% CI) for the levosimendan/placebo ratio of the number of VES/h.
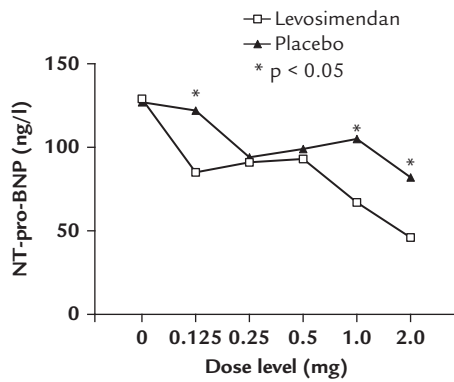
Figure 4. Median NT-pro-BNP values during the study.

level. One placebo patient had coronary insufficiency and angina pectoris reported as a serious adverse event.

Discussion

Low oral levosimendan doses induced an increase in cerebral blood flow velocities in our study. The cerebral blood flow was assessed using transcranial Doppler method, which is a surrogate for cerebral blood flow. Bravo et al[15] showed improved cerebral perfusion and oxygenation with intravenous levosimendan in critically ill infants. Because levosimendan is a vasodilating agent that does not cause vasospasm in any arterial bed,[16] the likely explanation is a vasodilatory effect in the brain circulation. It is of importance that the effect was evident despite the fact that all patients were receiving an effective vasodilatory treatment with either ARB or angiotensin-converting enzyme inhibitor. Because there was no effect on the BHI, levosimendan did not seem to interfere with the carbon dioxide reactivity of cerebral arteries. The BHI levels were consistently fairly low in this population, which may be a confounding factor (ie, associated with greater arterial stiffness).

The antiaggregatory effects of levosimendan on platelets could also be of importance in stroke prevention. Although we did not study this, earlier data indicate that levosimendan has antiaggregatory effects.[10] Cilostazol has shown beneficial effect on secondary stroke prevention in clinics.[17] Its main mechanism of action is believed to be its antiaggregatory effects, but as a phosphodiesterase inhibitor, it also has vasodilatory effects.[17] Therefore, multiple mechanisms of both levosimendan and cilostazol could potentially underlie their eventual efficacy in stroke prevention.

Assessing the proarrhythmic potential of levosimendan was in a crucial role in our study. The occurrence of ventricular tachycardia has been shown to be a clinically relevant predictor of proarrhythmia.[18] However, its expected incidence was considered low in our small-scale study of patients without evident heart disease. We therefore decided to use the number of VES/h as a surrogate marker for proarrhythmia.

Earlier data indicate that a high frequency of VES/h is correlated with a worse prognosis. Mortality is higher in patients with myocardial infarction with a number of VES/h exceeding 30 compared with those with < 30 VES/h.[19] Furthermore, the occurrence of VES in a 2-minute ECG recording has been shown to be an independent risk factor for stroke in a large, community-based cohort of about 15,000 middle-aged subjects and a follow-up of up to 17 years. The hazard ratio for stroke was 1.7 in those with at least 1 VES in a 2-minute ECG (corresponding with ≥ 30 VES/h).[20]

There is no commonly accepted rule for a clinically relevant increase in VES/h to herald an increased risk of severe

proarrhythmic events. Further, the significance of the increase of VES as a predictor of proarrhythmic potential of a medication is even more limited. We hypothesized that a 2-fold increase in the number of VES/h could be of clinical importance. Only the highest dose—2.0 mg levosimendan daily—crossed this arbitrary line for proarrhythmia in our study.

Possible treatment effects on HR are also relevant for long-term safety, because elevated HR may increase myocardial oxygen consumption and thus be deleterious in ischemic conditions. Levosimendan did not influence the mean 24-hour HR at the 0.125- and 0.25-mg doses, whereas an increase of 5 to 9 beats/min was observed with the higher doses. For comparison, in the PERSIST study,[11] 1.0- and 2.0-mg doses of levosimendan resulted in an increase in HR of about 8 beats/min, which is similar to that observed in our study. The mechanism underlying the increase in HR is not known, but a direct increasing effect on sinus nodal rate has been suggested.[21]

In accordance with data in patients with heart failure,[11] levosimendan significantly decreased NT-pro-BNP in our study. The effect is probably related to vasodilation and decreased left ventricular filling pressure.[22] These effects, which are favorable in patients with heart failure, would possibly also be favorable in our study population. Patients with a history of previous stroke have a higher incidence of left ventricular dysfunction than matched controls.[23]

The pharmacokinetic properties of levosimendan induce certain limitations to constant oral dosing. Levosimendan has an elimination half-life of about 1 hour, but its metabolites, OR-1855 and OR-1896, have a half-life of about 70 to 80 hours in patients with heart failure.[24] The daily oral levosimendan dose must remain relatively low to avoid excessive accumulation of the metabolites. The metabolite OR-1896 has similar pharmacologic effects to the parent drug,[1] and in long-term oral treatment the clinical effects are mostly related to the active metabolite instead of levosimendan itself.[11] The 18-day dosing for each dose level in our study was selected to ensure that a steady-state level for the metabolites was reached by the end of the dosing period.

The plasma levels of the levosimendan metabolites OR-1855 and OR-1896 in our study were approximately half of those seen in patients with heart failure who were given oral levosimendan for 2 weeks.[13] The elimination half-life of the metabolites is considerably lower in healthy volunteers[25] than in patients with heart failure;[24] that is, 44 to 61 hours versus 70 to 80 hours, respectively. Although not measured in our study, the lower steady state of the metabolites suggests that the elimination half-life in a noncardiac stroke population is closer to that seen in healthy volunteers.

A major limitation of the study is the small number of patients included. Another limitation is the noncentralized reading of TCD imaging, although measures to harmonize the technical performance were taken.
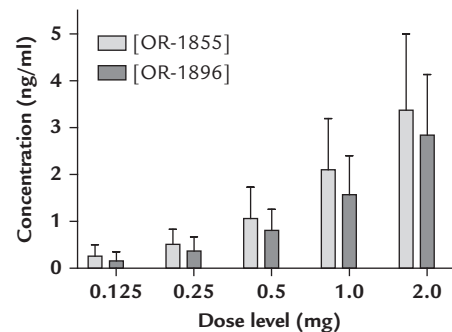

Figure 5. Mean (SD) plasma concentrations of levosimendan metabolites OR-1855 and OR-1896 at the end of each dosing period.

Conclusions

We showed that low oral levosimendan doses increase cerebral blood flow velocities and decrease NT-pro-BNP in patients with an earlier ischemic cerebrovascular event. Increased cerebral blood flow velocities were seen when levosimendan was administered as an add-on therapy to other drugs commonly used in secondary prevention of stroke. In preclinical models, levosimendan has shown beneficial outcome effects. However, it remains unknown whether the improved cerebral blood flow velocities induced by levosimendan have any clinical implications in the secondary prevention of stroke. On the whole, the preliminary safety profile was acceptable in this population; only the highest dose—2.0 mg daily—increased the number of VES/h compared with placebo. No episodes of atrial fibrillation or flutter were observed.

Acknowledgments

Conflicts of Interest

References

[1] Papp Z, Edes I, Fruhwald S, De Hert SG, et al. Levosimendan: Molecular mechanisms and clinical implications. Consensus of experts on the mechanisms of action of levosimendan. *Int J Cardiol.* 2012;23(159):82–87.

[2] Nieminen MS, Akkila J, Hasenfuss G, et al. Hemodynamic and neurohumoral effects of continuous infusion of levosimendan in patients with congestive heart failure. *J Am Coll Cardiol.* 2000;36:1903–1912.

[3] Packer M, Colucci W, Fisher L, et al. REVIVE Heart Failure Study Group. Effect of levosimendan on the short-term clinical course of patients with acutely decompensated heart failure. *JACC Heart Fail.* 2013;1:103–111.

[4] Slawsky MT, Colucci WS, Gottlieb SS, et al. Acute hemodynamic and clinical effects of levosimendan in patients with severe heart failure. *Circulation.* 2000;102:2222–2227.

[5] Mebazaa A, Nieminen MS, Packer M, et al. Levosimendan vs dobutamine for patients with acute decompensated heart failure: the SURVIVE Randomized Trial. *JAMA.* 2007;297:1883–1891.

[6] Levijoki J, Kivikko M, Pollesello P, et al. Levosimendan alone and in combination with valsartan prevents stroke in Dahl salt-sensitive rats. *Eur J Pharmacol.* 2015 [in press].

[7] Hein M, Zoremba N, Bleilevens C, et al. Levosimendan limits reperfusion injury in a rat middle cerebral artery occlusion (MCAO) model. *BMC Neurol.* 2013;13:106.

[8] Kersten JR, Montgomery MW, Pagel PS, Warltier DC. Levosimendan, a new positive inotropic drug, decreases myocardial infarct size via activation of KATP channels. *Anesth Analg.* 2000;90:5–11.

[9] Parissis JT, Karavidas A, Bistola V, et al. Effects of levosimendan on flow-mediated vasodilation and soluble adhesion molecules in patients with advanced chronic heart failure. *Atherosclerosis.* 2008;197:278–282.

[10] Kaptan K, Erinç K, Ifran A, et al. Levosimendan has anti-inhibitory effect on platelet function. *Am J Hematol.* 2008;83:46–49.

[11] Nieminen MS, Cleland JG, Eha J, et al. Oral levosimendan in patients with severe chronic heart failure –the PERSIST study. *Eur J Heart Fail.* 2008;10:1246–1254.

[12] Silvestrini M, Vernieri F, Pasqualetti P, et al. Impaired cerebral vasoreactivity and risk of stroke in patients with asymptomatic carotid artery stenosis. *JAMA.* 2000;283:2122–2127.

[13] Puttonen J, Laine T, Ramela M, et al. Pharmacokinetics and excretion balance of OR-1896, a pharmacologically active metabolite of levosimendan, in healthy men. *Eur J Pharm Sci.* 2007;32:271–277.

[14] Põder P, Eha J, Sundberg S, et al. Pharmacodynamics and pharmacokinetics of oral levosimendan and its metabolites in patients with severe congestive heart failure: a dosing interval study. *J Clin Pharmacol.* 2004;44:1143–1150.

[15] Bravo MdC López P, Cabañas F, et al. Acute Effects of Levosimendan on Cerebral and Systemic Perfusion and Oxygenation in Newborns: An Observational Study. *Neonatology.* 2011;99:217–223.

[16] Cengiz SL, Erdi MF, Tosun M, et al. Beneficial effects of levosimendan on cerebral vasospasm induced by subarachnoid haemorrhage: An experimental study. *Brain Injury.* 2010;24:877–885.

[17] Qian Y, Bi Q. Systematic study of cilostazol on secondary stroke prevention: a meta-analysis. *Eur J Med Res.* 2013;18:53.

[18] de Sousa MR, Morillo CA, Rabelo FT, et al. Non-sustained ventricular tachycardia as a predictor of sudden cardiac death in patients with left ventricular dysfunction: A meta-analysis. *Eur J Heart Fail.* 2008;10:1007–1014.

[19] Hedblad B, Janzon L, Johansson BW, Juul-Moller S. Survival and incidence of myocardial infarction in men with ambulatory ECG-detected frequent and complex ventricular arrhythmias. 10 year follow-up of the 'Men born 1914' study in Malmo, Sweden. *Eur Heart J.* 1997;18:1787–1795.

[20] Agarwal SK, Heiss G, Rautaharju PM, et al. Premature ventricular complexes and risk of incident stroke: the Atherosclerosis Risk in Communities (ARIC) Study. *Stroke.* 2010;41:588–593.

[21] Toivonen L, Viitasalo M, Sundberg S, et al. Electrophysiologic effects of a calcium sensitizer inotrope levosimendan administered intravenously in patients with normal cardiac function. *J Cardiovasc Pharmacol.* 2000;35:664–669.

[22] Stolker JM, Rich MW. Clinical utility of B-type natriuretic peptide for estimating left ventricular filling pressures in unselected elderly patients undergoing diagnostic coronary angiography. *J Invasive Cardiol.* 2010;22:107–112.

[23] Hays AG, Sacco RL, Rundek T, et al. Left ventricular systolic dysfunction and the risk of ischemic stroke in a multiethnic population. *Stroke.* 2006;37:1715–1719.

[24] Kivikko M, Antila S, Eha J, et al. 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;40:465–471.

[25] Antila S, Pesonen U, Lehtonen L, et al. Pharmacokinetics of levosimendan and its active metabolite OR-1896 in rapid and slow acetylators. *Eur J Pharm Sci.* 2004;23:213–222.