EFFECTS OF LEVOSIMENDAN ON CELLULAR METABOLIC ALTERATIONS IN PATIENTS WITH SEPTIC SHOCK: A RANDOMIZED CONTROLLED PILOT STUDY

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ABSTRACT—Introduction: Mitochondrial dysfunction and consequent cellular energetic failure play a key role in the development of sepsis-related organs failure. Evidence suggests that the pleiotropic effects of levosimendan may positively affect cellular metabolism during septic shock. Objectives: To investigate changes in the concentration of glucose, lactate, pyruvate, and glycerol in the extracellular fluid of the skeletal muscle following levosimendan administration in patients with septic shock. Methods: The study was designed as a prospective, double-blind, controlled, clinical pilot trial and performed in a multidisciplinary intensive care unit. After achieving normovolemia and a mean arterial pressure of at least 65 mm Hg, 20 septic shock patients were randomized to receive either levosimendan 0.2 μg/kg/min (n = 10), or dobutamine 5 μg/kg/min as active comparator (n = 10). Interstitial tissue concentrations of lactate, pyruvate, glucose, and glycerol were obtained by using muscle microdialysis. All measurements, including data from right heart catheterization, were obtained at baseline and every 6 h for the following 72 h after randomization. The trial is registered with Clinicaltrials.gov, number NCT02963454.

Results: Compared with dobutamine, levosimendan increased interstitial tissue pyruvate concentration (153.3 ± 73 and 187.2 ± 13.5 vs. 210.7 ± 66.2 and 161 ± 64.6; P < 0.05), and lactate clearance (55 vs. 10). Lactate/pyruvate ratio was lower in the levosimendan group at the end of study period (37.7 ± 18.9 and 29.3 ± 12.7 vs. 10.9 ± 4.5 and 31.4 ± 13.2; P < 0.05).

Conclusion: Although we investigated a small number of patients, our preliminary results suggest that levosimendan may improve cellular metabolic alterations in patients with septic shock.

KEYWORDS—Levosimendan, metabolism, microcirculation, microdialysis, septic shock

INTRODUCTION

Cellular energetic failure often characterizes the progression of septic shock and is associated with increased mortality (1–4). The underlying mechanisms of such energetic failure are complex and involve several pathophysiological pathways including microvascular derangement and mitochondrial dysfunction (1–4). Reversing cellular energetic failure may represent an effective therapeutic target for improving outcome. Evidence suggests that levosimendan can be safely administered in patients with septic shock requiring inotropic support (5). However, when used to prevent organ dysfunction, levosimendan did not show outcome benefit (6). In addition to its inotropic and vasodilator effects, levosimendan (a calcium sensitizer), has anti-inflammatory, antiapoptotic and antioxidant effects (7). Furthermore, it may positively affect mitochondrial function by opening adenosine triphosphate (ATP)-sensitive potassium (KATP) channels (7). Due to these pleiotropic properties, levosimendan may potentially improve cellular metabolism. Nevertheless, the effects of this drug on cellular metabolic activity in septic shock are not fully elucidated. Microdialysis allows the analysis of the interstitial fluid concentrations of glucose, lactate, pyruvate, and glycerol which reflect the energy metabolism of the cells (8, 9). Indeed, it is widely accepted that lactate/pyruvate ratio (L/P ratio) is a marker of the adequacy of oxygen delivery to tissues oxygen demand and correlates with clinical outcome (8, 9). Microdialysis may thus be used to accurately detect the efficacy of pharmacological interventions in improving cellular metabolism. The aim of the present randomized, double-blind, controlled pilot study was therefore to investigate the effects of levosimendan on cellular metabolism in patients with septic shock, by using muscle microdialysis. We hypothesized that levosimendan improves interstitial L/P ratio and lactate clearance.

PATIENTS AND METHODS

Patients

After the approval by the Institutional Ethics Authorities, the study was performed in the intensive care unit of the Tunis Military Hospital and registered in Clinicaltrials.gov as NCT02963454. Written informed consent was obtained from the patients’ next of kin or from the legally authorized representatives. Enrolment of patients started in August 2011 and ended in May 2014. We enrolled patients who fulfilled the criteria of septic shock requiring norepinephrine (NE) to maintain a mean arterial pressure (MAP) of at least 65 mm Hg despite appropriate volume resuscitation (pulmonary...
arterial occlusion pressure \([PAOP] = 12 \text{ mm Hg}\) to 18 mm Hg and central venous pressure \([CVP] = 8 \text{ mm Hg}\) to 12 mm Hg\) (10). Septic shock criteria were defined according to current Surviving Sepsis Campaign guidelines (10) which include the presence of sepsis-related refractory hypotension \((\text{MAP} < 65 \text{ mm Hg})\) unresponsive to fluid challenge \((20 \text{ mL/kg}–40 \text{ mL/kg})\). Exclusion criteria were pregnancy, uncontrolled hemorrhage, terminal heart failure, significant valvular heart disease, documented or suspected acute coronary syndrome, and limitations on the use of inotropes: left ventricle outflow obstruction, systolic anterior motion of the mitral valve.

**Systemic hemodynamics, global oxygen transport, and acid–base homeostasis**

Systemic hemodynamic monitoring of the patients included a pulmonary artery catheterer \((7.5-	ext{F}; \text{ Edwards Lifesciences, Irvine, Calif})\) and a radial artery catheterer. MAP, CVP, mean pulmonary arterial pressure, and PAOP were measured at end-expiration. Heart rate was analyzed from a continuous recording of electrocardiography with ST segments monitored. Cardiac index was measured using the continuous thermodilution technique (Vigilance II; Edwards Lifesciences). Oxygen delivery index \((DO_2)\), oxygen consumption index, and oxygen extraction ratio were calculated by means of standard formulae.

Arterial and mixed-venous blood samples were collected to determine oxygen tensions and saturations as well as carbon dioxide tensions, standard bicarbonate, \(pH\), lactate concentrations, glucose, and hemoglobin.

**Microdialysis variables**

Muscle microdialysis was performed using a microdialysis probe \((\text{ CMA 60, CMA/microdialysis, Stockholm, Sweden})\) inserted into the quadriceps femoris muscle and connected to a microinjection pump \((\text{ CMA 107, CMA microdialysis AB, Solna, Sweden})\) at 0.3 \(\mu\text{L/min}\) of perfusion flow rate of ringer solution without lactate \((8, 11, 12)\). The sampling of the dialysate liquid was started after a 2 h equilibration period \((13)\). Microrcal tubes for collecting samples of the interstitial fluid were collected concomitantly with the hemodynamic evaluation. The interstitial tissue concentrations of lactate, pyruvate, glucose, and glycerol were analyzed by using a CMA 600 Analyser \((\text{ CMA 600 Microdialysis Analyser; CMA Microdialysis AB})\). The L/P ratio was calculated automatically by the machine. As previously described \((14)\), both blood and muscle lactate clearance were defined by the equation: \([\text{lactate}_{\text{baseline}} – \text{lactate}_{\text{delayed}}]/\text{lactate}_{\text{baseline}} \times 100\%\). Lactate delayed are blood lactate concentrations measured at 12, 24, 48, and 72 h. Positive values indicate a decrease or clearance of lactate \((14)\).

**Study design**

Twenty patients were randomly allocated to the treatment with either intravenous levosimendan 0.2 \(\mu\text{g/kg per minute}\) for 24 h or intravenous dobutamine 5 \(\mu\text{g/kg per minute}\) as active comparator, in a double-blinded manner \((\text{each n} = 10)\). Dobutamine were administered during all the 72 h study period. During the study period, conventional treatment was continued as per usual practice. Fluid challenges were performed, and repeated as necessary, to maintain \(CVP > 8 \text{ mm Hg}\) and \(PAOP > 12 \text{ mm Hg}\) \((10)\). NE was titrated to maintain \(\text{MAP} > 65 \text{ mm Hg}\). Packed red blood cells were transfused when \(\text{Hb}\) concentrations decreased below \(7 \text{ g/dL}\), or if the patient exhibited clinical signs of inadequate systemic oxygen supply \((10)\). All patients were sedated with remifentanil and midazolam and received mechanical ventilation using a volume-controlled mode. All measurements including systemic hemodynamic variables, acid–base variables blood gases, and glucose as well as interstitial tissue concentrations of lactate, pyruvate, glucose, and glycerol were determined at baseline and then every 6 h for the following 72 h after randomization.

**Statistical analysis**

Statistical analysis was performed by using SPSS v.20.0 \((\text{ IBM Corp, Armonk, NY})\) software for statistical analysis. Baseline and demographic data were compared with a Mann–Whitney rank sum test or \(t\)-square test, as appropriate. Microvascular and hemodynamic variables were analyzed with a \(t\)-Mann–Whitney rank sum test. Data are expressed as median \((25\text{th}; 75\text{th percentile})\), if not otherwise specified. Correlations between arterial and interstitial lactate and glucose concentrations within each group were analyzed by Spearman rank order correlation. A \(P\) value < 0.05 was considered statistically significant. As a pilot study, our aim was to provide preliminary data to test the hypothesis that levosimendan may affect cellular metabolic alterations in patients suffering from septic shock. Due to the lack of clinical data, the analyses performed in the present study were mandatory exploratory and no \(a\) \textit{ex ante} power analysis was performed. Nevertheless, \(a\) \textit{posteriori} calculation, based on the primary results of the study, indicates that a sample size of eight subjects per group would have 80% power to detect a difference of 5% between groups using a paired \(t\) test at the 95% significance level.

**RESULTS**

**Demographic data**

Thirty-two patients with septic shock were screened and 20 patients were included in the study \((\text{Fig. 1})\). Demographic characteristics including age, gender, medical history, site of infection, Simplified Acute Physiology Score II score, and mortality were not different among groups \((\text{Table 1})\).

**Systemic hemodynamics, oxygen transport variables, acid–base homeostasis, and blood glucose**

Systemic hemodynamic variables, NE requirements, fluids administered, blood glucose, and acid–base homeostasis were

**TABLE 1. Demographic characteristics of the investigated patients**

|                | Control \((n = 10)\) | Levosimendan \((n = 10)\) | \(P\)  |
|----------------|----------------------|---------------------------|--------|
| Age, years     | 61 [24–69]           | 51 [36–62]                | 0.469  |
| Males \((n)\)  | 9                    | 8                         | 0.535  |
| SAPS II        | 51 [48–68]           | 57 [44–66]                | 0.305  |
| Comorbidities \((n)\) |                      |                           |        |
| Diabetes mellitus | 4                    | 3                         | 0.861  |
| Dyslipidemia   | 3                    | 1                         | 0.475  |
| Hypertension   | 5                    | 3                         | 0.563  |
| Sepsis sites \((n)\) |                      |                           |        |
| Pulmonary      | 5                    | 4                         |        |
| Abdominal      | 2                    | 1                         |        |
| Central venous catheter | 1                | 1                         | 0.562  |
| Endocarditis   | 1                    | 1                         |        |
| More than two sites | 1                   | 3                         |        |
| Mortality \((\%)\) | 50                   | 30                        | 0.608  |

Data are presented as median \((25\text{th}; 75\text{th percentile})\) or percentage. SAPS II indicates simplified acute physiology score.
Hemodynamic variables

| Variables         | Groups       | Baseline | 24 h      | 48 h      | 72 h      |
|-------------------|--------------|----------|-----------|-----------|-----------|
| Heart rate (bpm)  | Control      | 92 [84–102] | 101 [90–112] | 105 [92–121] | 94 [82–108] |
|                   | Levo         | 102 [80–110] | 108 [90–116] | 108 [82–118] | 103 [81–112] |
| MAP (mm Hg)       | Control      | 73 [62–86] | 80 [69–91] | 78 [67–85] | 73 [60–87] |
|                   | Levo         | 74 [60–84] | 77 [65–91] | 77 [60–88] | 81 [74–94] |
| CI (l/min/m²)     | Control      | 3.5 [3.2–4.4] | 4.1 [3.7–4.9] | 4.2 [3.8–5.1] | 4 [3.1–4.9] |
|                   | Levo         | 4 [3–4.6] | 4.5 [3.6–5.2] | 4.4 [3.8–5.4] | 4.7 [3.9–5.2] |
| CVP (mm Hg)       | Control      | 10.2 [9–11.1] | 9.2 [8.1–11.9] | 10.5 [9–12.9] | 10 [9.3–12.1] |
|                   | Levo         | 9.5 [8.2–10.9] | 10.3 [9–12.1] | 12.1 [10–13.4] | 11 [10.3–12.4] |
| VO₂I (ml/min/m²)  | Control      | 452 [387–532] | 539 [402–631] | 623.9 [567–730] | 538.8 [497–602] |
|                   | Levo         | 546 [497–646] | 594 [477–652] | 588 [497–629] | 611 [507–694] |
| VO₂-ER (%)        | Control      | 143 [87–212] | 156 [94–282] | 161 [94–302] | 149 [76–227] |
|                   | Levo         | 151 [71–231] | 167 [80–272] | 159 [77–287] | 141 [81–294] |
| O₂-ER (%)         | Control      | 26 [20–34] | 25 [20–32] | 26 [21–35] | 28 [21–37] |
|                   | Levo         | 24 [19–32] | 27 [22–33] | 25 [19–38] | 28 [18–36] |
| NE Dose (μg/kg/min) | Control     | 0.2 [0.1–0.7] | 0.27 [0.1–0.6] | 0.25 [0.1–0.8] | 0.29 [0.08–0.9] |
|                   | Levo         | 0.3 [0.1–0.8] | 0.34 [0.2–0.9] | 0.3 [0.18–1.1] | 0.29 [0.1–1] |
| Glycemia (mmol/L) | Control      | 12 [11.1–12.8] | 10.3 [9–11.8] | 10.8 [9.1–12.9] | 10.1 [9.2–11.8] |
|                   | Levo         | 10.7 [9.1–12.4] | 11.8 [10.1–12.9] | 10.4 [8.9–12.4] | 9.7 [8.7–10.9] |
| Fluid input, mL/24 h | Control   | 1,010 [902–1,220] | 898 [778–1,120] | 895 [778–1,129] | 890 [778–1,101] |
|                   | Levo         | 1,185 [1,078–1,328] | 997 [842–1,200] | 912 [808–1,190] | 909 [838–1,124] |

Data are presented as median [25th; 75th percentile]. No statistically significant differences were found in any of the investigated variables.

**Microdialysis variables**

At baseline, the interstitial tissue concentrations of lactate, pyruvate, glucose, glycerol, as well as L/P ratio did not differ between groups. However, at the end of the study period we noticed a significant decrease in the muscle L/P ratio in the levosimendan group (28.4 [20.4–45.5] vs. 10.1 [6.9–14.4] respectively; P = 0.001). Muscle lactate tended to be lower in the levosimendan group at the end of the study period. However, this difference did not reach statistical significance (P = 0.05). No statistically significant differences were found in the other investigated variables.

Negative weak correlations were found between DO₂I and muscle lactate (rho = −0.234, P < 0.001), L/P ratio (rho = −0.142, P = 0.021), and muscle glucose (rho = −0.309, P < 0.001).

A significant positive correlation existed between muscle and blood lactate concentrations and between muscle and blood glucose concentrations.

**DISCUSSION**

This pilot study was the first to evaluate changes in the concentrations of glucose, lactate, pyruvate, and glycerol in the cells at different time points (Tables 2 and 3).
extracellular fluid of the skeletal muscle during levosimendan administration in patients with septic shock. The major finding of the present study is that levosimendan improved cellular energy metabolism, as indicated by reduced L/P ratio and both increased lactate clearance and pyruvate concentration. Of note, such improvement was not related to changes in systemic hemodynamics.

Interstitial L/P ratio is influenced by the cellular switching from aerobic to anaerobic energy metabolism and thus indicates the presence of tissue local oxygen delivery and consumption mismatching (9, 12, 13). Indeed, a decreased oxygen and glucose cellular availability leads to anaerobic energy metabolism with increased lactate and reduced pyruvate concentrations as pyruvate is metabolized to lactate. As a consequence, under hypoxic conditions L/P ratio increases (9, 12, 13). Changes in L/P ratio may therefore accurately indicate a condition of tissue hypoxia and/or ischemia, and it is widely accepted that a ratio >25 indicates the onset of anaerobic metabolism (9, 14). Nevertheless, an increased L/P ratio in the presence of elevated pyruvate may be the consequence not only of decreased oxygen delivery (ischemic hypoxia) but also of mitochondrial dysfunction (9, 15).

In the present study, we found elevated baseline values of L/P ratio and pyruvate in both dobutamine and levosimendan groups. These findings indicate that despite the achievement of adequate hemodynamic optimization, our patients suffered from a condition of emerging cellular energetic failure.

Levosimendan is a calcium-sensitizing drug and exerts its inotropic effect principally via binding to the calcium-saturated troponin C of myocardial thin filament. This action results in stabilization of the calcium-bound conformation of troponin, thereby prolonging the actin–myosin interaction without altering cross-bridge cycling. In addition, levosimendan exerts vasodilatory properties via the activation of ATP-dependent potassium channels (KATP) (5–8).

In patients with septic myocardial dysfunction levosimendan improves myocardial performance (16–18), exerts anti-inflammatory effects, and decreases iNOS expression and activity, as well as NF-kB-dependent transcription (7, 19). Furthermore, levosimendan improves both endothelial function and the endothelium-dependent flow-mediated dilatation (7). By opening the KATP channel located at the level of mitochondria, levosimendan may also preserve cellular energy homeostasis and protect mitochondria from oxidative stress (7, 19–21).
Taken together, all these findings suggest that the pleiotropic effects of levosimendan may positively affect sepsis-induced cellular metabolic alterations. In fact, we observed that levosimendan attenuated cellular energetic failure as indicated by reduced L/P ratio at the end of study period. The reduction in L/P ratio indicates that the improvement in energy metabolism was due to increased cellular oxygen availability. This assumption is strengthened by the parallel increase in pyruvate concentration following levosimendan administration. Such an increase can be explained with the recovery of aerobic metabolism which led to accelerated cellular uptake and conversion of lactate into pyruvate by lactate dehydrogenase for increased oxidation (22).

At the level of microcirculation, sepsis induces a reduction of the perfused vessel density, leading to augmented intercapillary distances. As a result, the oxygen diffusion distance increases and may exceed the critical threshold (the maximum distance from oxygen source that allows the maintenance of mitochondrial efficiency).

Thanks to its vasodilatory and pleiotropic effects, levosimendan enhances both convection and diffusion (increased perfused vessel density), thereby reducing oxygen diffusion distance. This in turn increases microvascular oxygen delivery and cellular oxygen availability (23, 24).

We cannot exclude that a better mitochondrial performance contributed to such improvement. In this regard, it has been shown that levosimendan did not affect the activity of the respiratory chain complexes I, II, and III in a small series of septic shock patients (22). However, levosimendan, by opening mitochondrial KATP channels, had the ability to protect mitochondria from the excessive production of reactive oxygen species, such as superoxide, peroxynitrite, and nitric oxide and this ability may potentially mitigate the bioenergetic failure (7, 21, 22). From a pathophysiological point of view, an improvement in microvascular blood flow precedes the recovery of the cellular metabolic alterations and thus the reversal of bioenergetic failure. This may explain why we observed improved cellular metabolism after 72 h from levosimendan administration. However, the present study design did not allow us to assess the time frame relationships between changes in cellular metabolism and microvascular blood flow, as investigating the latter requires different monitoring techniques (25).

In accordance with the previous study (24), our protocol required the administration of 5 μg · kg⁻¹ · min⁻¹ dobutamine as an active comparator and such dobutamine dose did not affect neither systemic hemodynamics nor microdialysis variables. In this regard studies on the effects of dobutamine on microcirculation show conflicting findings. De Backer et al. (26) demonstrated that dobutamine at the dose of 5 μg · kg⁻¹ · min⁻¹ improved but failed to normalize capillary blood flow in patients with septic shock. By contrast, in a more recent study, the administration of similar dobutamine dose failed to improve microcirculation (27). Adrenergic receptor and signaling abnormalities that typically occur during septic shock may account for the heterogeneous response of microcirculation following dobutamine administration.

The present study has some limitations that we would like to acknowledge. First, due to limited human and economic resources the number of investigated patients was small. However, the sample size was similar to previous experimental and clinical studies with the respective methodology (28–31) and the consistency of the cellular metabolic response to levosimendan was evident. Second, owing to the lack of investigation of specific mitochondrial variables such as the activities of the respiratory chain complexes, we cannot conclude whether improved mitochondrial function has contributed to the observed changes in cellular energy metabolism. Third, we investigated the changes in the metabolism of the muscle cells which might not be representative of the cells in other tissues. Fourth, in accordance with the previous study (24), we used a low dose of dobutamine as an “active comparator” to facilitate blinding of the study drugs as levosimendan may induce evident hemodynamic changes. Because the aim of our study was not to perform a direct comparison between the two drugs, a critical discussion on the effect of dobutamine is outside the scope of the present study. Fifth, patients receiving levosimendan were younger (although not significantly different), therefore we cannot exclude age-related different effects of the study drug. Nevertheless, systemic hemodynamic and acid–base homeostasis did not differ between the two study groups. Finally, our study protocol required a 72 h observational period. Such a study design does not allow excluding a direct time-dependent effect unrelated to the specific agent.

**CONCLUSION**

This is the first prospective, randomized clinical study investigating the effects of levosimendan on cellular metabolism in septic shock patients by analyzing the concentrations of glucose, lactate, pyruvate, and glyceral in the extracellular fluid of the skeletal muscle. Although we investigated a small number of patients, our results demonstrate that levosimendan at 0.2 μg/kg per minute improves muscle cellular energy metabolism in volume-resuscitated septic shock patients. Such improvement was mainly the consequence of increased oxygen delivery at the level of microcirculation as it was not associated with changes in systemic hemodynamics. These results need to be confirmed in a larger trial.

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