The Role of Calcium Desensitization in The Pathophysiology of Septic Myocardial Depression and Effects of Levosimendan:: Results of Fifteen Septic Shock Patients

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Abstract
Decreased global contractility is relatively common in patients with septic shock. Levosimendan is a novel inodilator that improves cardiac contractility by sensitizing troponin C to calcium. Calcium desensitization plays an important role in the pathophysiology of septic myocardial depression. For these reasons, we thought that levosimendan might be beneficial to the patients with sepsis-induced cardiac dysfunction. We infused levosimendan for 24 hours to 15 septic shock patients after 24 hour of conventional treatment (dopamine or adrenaline) and observed mean urine output, mean arterial pressure, changes of conventional inotropic agents' dose, central venous pressure and outcome. In nine (%70) of the fifteen patients, infusion of conventional inotrops have been decreased or ceased. Nine patients (%70) were died, but others (%30) were recovered from shock. Nine patients died and 3 of them died at 96 hours after levosimendan infusion. Urine outputs of all patients have been adequate. In only six (%40) patients, no urine output has been observed. Our series precede that levosimendan can be used in hopeless and unsuccessfully treated decompensate myocardial failure of septic shock. Therefore, we suggested that levosimendan, removes the symptoms of acute cardiac failure in septic shock and improves the hemodynamic function, in addition to slow down or stop the progressive ventricle dysfunction and to form a hemodynamic balance and improve the survival. As our impression, levosimendan may be an alternative to the strategy of increasing the dose of conventional inotrops and unsuccessfully treated decompensate myocardial failure under septic shock status.

INTRODUCTION
In septic shock, the hemodynamic profile is characterized by components of hypovolemic, obstructive, cardiogenic, distributive, and cytotoxic shock (1). After fluid therapy, the presence and severity of hypotension are directly dependent on the impairment of contractility and the degree of systemic vascular resistance decrease (2, 3). Persistent hypotension, despite adequate fluid resuscitation, mandates the use of vasopressors and is the hallmark of septic shock. But, decreased global contractility is relatively common in patients with septic shock. Myocardial depression expressed as biventricular decreased ejection fraction and dilatation typically peaks within the first days of septic shock and returns to normal values in survivors within 10 days (4, 5). This sepsis-induced myocardial depression is made by cytokines probably via altering intracellular calcium homeostasis in the cardiomyocyte (6, 7). Tavernier et al. (8) showed in an experimental model of sepsis that cardiac mitochondrial and creatine kinase systems remain unaltered, whereas protein phosphorylation decreases myofibrillar Ca^{2+} sensitivity and may contribute to the depression of cardiac contractility. The reduced myofilament Ca^{2+} sensitivity is also associated with increased length in single cardiac myocytes and may cause the acute ventricular dilation observed during sepsis (9, 10). In addition, increasing blood pressure through vasoconstrictor drugs may result in a decrease in cardiac output.

In the presence of severe depression of cardiac contractility and decreased cardiac output, inotropic therapy may be considered in an attempt to maintain a normal range of cardiac output. Levosimendan is a new inodilator that improves cardiac contractility by sensitizing troponin C to calcium. This drug has proved to be effective in treating advanced congestive heart failure and cardiogenic shock. In
patients with severe, low-output heart failure, levosimendan improved hemodynamic performance more effectively than dobutamine. Pretreatment with levosimendan in experimental hypo-dynamic septic shock in pigs has shown valuable effects in oxygen transport. Furthermore, Morelli et al showed that levosimendan improves systemic hemodynamic and regional perfusion in patients with septic shock.

Another effect of levosimendan is vasodilatation via activating adenosine triphosphate-regulated potassium (K\text{ATP}) channels. Renal perfusion may be expected to improve with its vasodilatory properties and urine output may be increased.

In preclinical and clinical studies, levosimendan has been shown to exert potent dose-dependent positive inotropic and vasodilatory activity and has emerged as a promising alternative to conventional inotropic agents for patients with decompensated heart failure. For these reasons, we thought that levosimendan might also have beneficial effects in sepsis-induced cardiac dysfunction unresponsive to standard inotropic and vasoconstrictor therapy.

**MATERIALS AND METHODS**

This study was a retrospective, observational evaluation. In patients with septic shock and heart failure, who used vasoconstrictive or inotropic agents, we used levosimendan if no response was seen to these agents. We evaluated the results of the 15 patients who used levosimendan retrospectively from intensive care unit records.

We used levosimendan in fifteen septic shock patients with cardiac failure unresponsive to standard treatment. In all cases despite anti-biotherapy, mechanical ventilation, fluid loading, strict glucose control, activated protein C, continuous infusion of vasopressors and corticosteroid therapy, a severe hypo-dynamic state was present. Myocardial competence was getting worse with anuria, increased adrenalin and β-adrenergic agonists' dose and severe increased mean arterial pressure. In our ICU, 15 septic shock patients received conventional treatment including dopamine or adrenalin for 24 hours and then 0.2 µg/kg/min levosimendan was infused continuously for 24 hours as they were taking intensive medical treatment. Their mean urine output, mean arterial pressure (MAP), changes in conventional inotropic agents' dose, central venous pressure (CVP) and outcomes were followed.

**STATISTICAL ANALYSIS**

For statistical analysis of numerical variables, nonparametric dependent groups were compared with Wilcoxon Signed Ranks Test, categorical variables with Fisher's Exact Test. Numerical variables were expressed as mean ± SD and categorical ones with number and percents. SPSS 10.0 for Windows was used for statistical analysis with 95% confidence interval and p<0.05 was accepted as significant level.

**RESULTS**

The demographic characteristics and classification according to APACHE II scores of the patients are shown in Table 1.

**Figure 1**

Table 1: Diagnosis, age, sex characteristics and APACHE II scores of the patients.(F: female, M: male)

<table>
<thead>
<tr>
<th>Case No</th>
<th>Diagnosis at follow-up in ICU</th>
<th>Age (year)</th>
<th>Sex</th>
<th>APACHE II Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Intra abdominal sepsis after operated for stomach cancer</td>
<td>63</td>
<td>F</td>
<td>32</td>
</tr>
<tr>
<td>2</td>
<td>Intra abdominal sepsis</td>
<td>47</td>
<td>M</td>
<td>22</td>
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<td>3</td>
<td>Penicillio due to bile leak</td>
<td>75</td>
<td>F</td>
<td>21</td>
</tr>
<tr>
<td>4</td>
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<td>76</td>
<td>F</td>
<td>18</td>
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<tr>
<td>9</td>
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<td>F</td>
<td>22</td>
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<td>10</td>
<td>Diabetic foot</td>
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<td>Operated for biliary cancer</td>
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</tr>
<tr>
<td>15</td>
<td>Intra abdominal sepsis after colon perforation</td>
<td>74</td>
<td>M</td>
<td>26</td>
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</table>

Average age was 65.40 ± 10.34 (between 45-80 years) and Average APACHE-II score was 23.47 ± 6.9 (between values 11-33) in our 15 patients. In nine (%70) of them, infusion of conventional inotrops have been decreased or ceased. Nine patients (%70) were died, but others (%30) were recovered from shock (Table 2 and 3).
Figure 2
Table 2: Urine output, MAP, CVP. Urine output (cc kg h⁻¹), MAP (mm Hg), CVP (cm H₀), before and after 24h levosimendan infusion.

<table>
<thead>
<tr>
<th>Case No</th>
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<th>Urine output after levosimendan</th>
<th>MAP before levosimendan</th>
<th>MAP after levosimendan</th>
<th>CVP before levosimendan</th>
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Figure 3
Table 3: Inotrop dose and outcome. Inotrop dose and clinical result status of our patients using conventional inotrop doses before and after 24h levosimendan infusion (D: dopamine µg.kg⁻¹.min⁻¹, A: adrenaline mg.h⁻¹).

Three of 9 dead patients, were died at 96 hours after levosimendan infusion. Urine outputs of all patients have been adequate. In only six (%40) patients, no urine output has been observed. Urine output (cc h⁻¹) increased in 83.3% of surviving and in 55.6% of dead patients after levosimendan infusion, but the difference was not statistically significant (p>0.05) (Figure 1).
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Figure 4
Figure 1: Urine output

Urine output (cc h⁻¹) increased in 83.3% of surviving and in 55.6% of dead patients after levosimendan infusion but the difference was not statistically significant (p>0.05). This difference can be statistically significant if larger series are studied.

CVP (cm H₂O) decreased in 83.3% of survived and 44.4% of dead patients after the drug but the differences were not statistically significant (Figure 2).

Figure 5
Figure 2: CVP values

CVP (cm H₂O) decreased in 83.3% of survived and 44.4% of dead patients after the drug but the difference was not statistically significant. If study is repeated with larger populations this difference can be significant.

For the last two tables (Figure 1 and 2) these differences can be statistically significant if larger series are studied. When average MAP values (mm Hg) before levosimendan infusion were compared with after levosimendan values, there was not any statistically significant difference (p>0.05) (Figure 3).

Figure 6
Figure 3: MAP values

Average MAP values (mm Hg) before and after levosimendan infusion were compared. There was not any statistically significant difference (p>0.05).

There was a statistically significant difference between the Dopamine (D) infusion dose (µg.kg⁻¹.min⁻¹) values before and after levosimendan infusion (p<0.05). Low average Dopamine value after the drug was the reason of the difference (Figure 4).

Figure 7
Figure 4: Changes of conventional inotropic agents' dose

There was a statistically significant difference between the Dopamine (D) infusion dose (µg.kg⁻¹.min⁻¹) (values before and after levosimendan infusion (p<0.05). Low average Dopamine value after the drug was the reason of the difference.

DISCUSSION

Ascertaining the incidence of septic shock is limited by the variability in definitions used in epidemiologic studies, the analysis of septic shock as a subset of patients with severe sepsis, and short-comings of methods used to calculate the incidence of severe sepsis (1). The incidence of septic shock ranged from 52% to 71% of patients with severe sepsis, with a mean 58% (1, 16–17). A recent study estimated 751,000 cases of severe sepsis per annum in the United States (17).
The mortality of septic shock can be estimated more reliably about %87 (1, 2).

In 1992, the ACCP/SCCM Consensus Conference Committee defined septic shock as follows: “...sepsis-induced hypotension (systolic blood pressure<90mm Hg or a reduction of >40mm Hg from baseline) despite adequate fluid resuscitation along with the presence of perfusion abnormalities that may include, but are not limited to, lactic acidosis, oliguria, or an acute alteration in mental status. Patients who are receiving inotropic or vasopressor agents may have a normalized blood pressure at the time that perfusion abnormalities are identified” (1, 2). This definition has received general acceptance with the exception that most clinical trials have not considered inotropic therapy alone as a qualifier for sepsis-induced cardiovascular failure. Most patients who remain hypotensive after volume resuscitation will be started on vasopressors; vasopressors requirement for sepsis-induced hypotension plus hypo perfusion abnormalities becomes a clinically useful surrogate definition for septic shock (1). β-adrenergic agonist (dopamine) has been demonstrated to be effective in raising blood pressure in patients with septic shock (1, 3).

Because of the rapid changes in blood pressure that may occur in the presence of septic shock, arterial cannulation for continual monitoring of blood pressure is recommended. In addition, central venous catheters are needed to infuse vasopressors. The role of central hemodynamic monitoring is less clear. It is now well established that use of the pulmonary artery catheter (PAC) is frequently associated with inaccurate measurements. Furthermore, even when measurements are accurate as us, benefit could only be gained when appropriate decisions are made based on these measurements (1, 2).

Decreased contractility is a major abnormality of septic myocardial depression, although a decrease in myocardial compliance may also play a role (3, 22). Among several factors proposed to cause sepsis-induced myocardial depression, cytokines play a major role probably by altering intracellular calcium homeostasis in the cardiomyocyte (6, 7, 13). The failing myocardium is a major source of pro-inflammatory cytokines (13, 24). Administration of lipopolysaccharide (LPS) has been shown to impair cardiac contractility and the contractile response to β-adrenergic stimulation by nitric oxide-cyclic guanosine monophosphate mediated decrease in myofilament responsiveness to Ca++ (13). As decreased myofilament responsiveness to Ca++ is a major determinant of septic myocardial depression, calcium sensitization may offer an attractive therapeutic approach to counteract septic myocardial depression, as already shown in animal experiments (13, 23). In addition, it has shown that compared with dobutamine, levosimendan increased intestinal blood flow and diminished intramucosal acidosis (26).

The calcium sensitizer levosimendan improves myocardial contractility by stabilizing troponin C and enhancing calcium sensitivity of cardiac myofilaments, however, it has no effect on myocardial oxygen demand and does not induce arrhythmias (11, 24, 27, 28). One advantage of levosimendan over catecholamine would be to increase the force of contraction without enhancing the influx of Ca++ into the cytosol and thus without increasing the risk of arrhythmias related to this ionic alteration (11, 21). Previous studies have demonstrated that levosimendan improves hemodynamic performance more effectively than β-adrenergic agonist in patients with severe heart failure, and it has been associated with improved long-term survival (11, 23). Although levosimendan has a long half-life, the improvement in cardiac output after a 24h intravenous infusion, has been shown to last for more than 7 days (23, 29). It was recently shown that levosimendan may decrease serum levels of pro-inflammatory cytokines and depress apoptotic process 48h after infusion (23, 29). Levosimendan decreased the pro-inflammatory cytokines: interleukin-6 (IL-6) and the apoptotic marker soluble FAS (sFAS) 24h after infusion, while this effect persisted for 7-30 days. However, tumor necrosis factor alpha (TNF-α) and soluble tumor necrosis factor receptors 1 (sTNF-R1) were decreased for a short period of time between 48h and 3d after infusion. sTNF-R2 was decreased 24h after infusion and remained lower than baseline for at least 7 days. These findings indicate that levosimendan depresses the expression of pro-inflammatory cytokines, soluble TNF receptors as well as sFAS circulating levels immediately after infusion, an effect which persists for 7-30 day (23). Levosimendan improves hemodynamic performance effectively and present a long-term prognosis in these patients (11, 23, 27, 28). Levosimendan could offer further therapeutic advantages in patients with advanced heart failure by improving systolic and diastolic right ventricular function (33) and left ventricular diastolic function (32-33).

Additionally, levosimendan-induced improvement of systolic function and peripheral vasodilatation may also attenuate peripheral tissue hypo perfusion leading to down-
regulation of cytokine extra cardiac production by transcriptional factors such as NF-kB (23, 24, 34). The improvement in hemodynamics could also decrease LPS release from congestive intestine, affecting NF-kB activation in peripheral blood leukocytes, as was recently proposed (23, 35).

By showing beneficial cardiac effects of levosimendan our results and other similar studies are in accordance with those reported in experimental sepsis studies (12, 13, 23). In the prospective experimental study with pigs of Oldner et al.; cardiac index and systemic oxygen delivery were markedly improved in the levosimendan group during endotoxemia. Systemic vascular resistance and blood pressure were reduced in the levosimendan group. Levosimendan also efficiently attenuated endotoxin-induced pulmonary hypertension. Portal venous blood flow and gut oxygen delivery were improved (13). The increase in left ventricular ejection fraction (LVEF) with levosimendan may be due to either to an increase in contractility or to a decrease in afterload (13). Arterial pressure, a major component of left ventricular afterload, did not change with levosimendan in Morelli study (13). Our result is same with these findings (figure 3).

Beneficial effects of levosimendan were at the renal level (13). In Morelli series; the increase in creatinine clearance with levosimendan could be related to a direct effect of the drug on renal perfusion since MAP was unchanged, and cardiac index increased in a smaller extent (13). In Pagel study; in endotoxemic animals levosimendan did not prevent the decrease in renal blood flow while protected against the decrease in cardiac output (13). However in the study of Oldner et al.; renal blood flow was unaffected, as was the endotoxin-induced increase in plasma endothelin-1-like immunoreactivity. In Morelli and this study the efficacy of levosimendan on renal function was confirmed the increase in urine output.

Although septic shock is associated with myocardial hypo responsiveness to catecholamine, one could anticipate that a higher dose of β-adrenergic agonist would have produced the same beneficial effects as levosimendan on hemodynamics (13). Morelli’s results suggest that levosimendan can exert beneficial effects in terms of systemic and regional hemodynamic in the setting of septic cardiac dysfunction after a 48h period of dobutamine administration and in their study's conclusion; these results suggest that levosimendan offers a safe and effective alternative to the strategy of increasing the dose of dobutamine in such a clinical setting. In our study; there is the absence of significant effect of adrenaline and another β-adrenergic agonist (dopamine) in cases septic-related cardiac dysfunction; levosimendan exert beneficial effects in urine output (figure 1);causes a decrease in afterload (increase in LVEF and no-change in MAP values-major component of LV afterload-figure 3), regulates fluid over-loading (decrease in CVP values at the discharged cases-figure 2) and decrease the doses of β-adrenergic agonist (dopamine-figure 4).

In conclusion; septic shock is a complicated, progressive and consequently lethal disease. In individuals with such a severe disease, cardiac contractility can be unstable or decompensate. Our series precede that levosimendan; by sensitizing myocardium to calcium and opening the potassium channels which are sensitive to ATP; can be used in hopeless and unsuccessfully treated decompensate myocardial failure of septic shock. We suggested that levosimendan removes the symptoms of acute cardiac failure in septic shock and improves the hemodynamic function, in addition to slow down or stop the progressive ventricle dysfunction and form a hemodynamic balance and improve the survival. As our impression, levosimendan may be an alternative to the strategy of increasing the dose of conventional inotrops and unsuccessfully treated decompensate myocardial failure under septic shock status.

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