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Effects of levosimendan in normodynamic endotoxaemia: a controlled experimental study☆,☆☆

Arnaldo Dubin*, Bernardo Maskin, Gastón Murias, Mario Omar Pozo, Juan Pablo Sottile, Marcelo Barán, Vanina Siham Kanoore Edul, Héctor Saúl Canales, Elisa Estenssoro

Cátedra de Farmacología, Facultad de Ciencias Médicas, Universidad Nacional de La Plata, 42 No. 577, 1900 La Plata, Argentina

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KEYWORDS
Septic shock;
Levosimendan;
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Summary
Objectives: Levosimendan is an inotropic and vasodilator drug that has proved to be useful in cardiogenic shock. Pretreatment with levosimendan in experimental hypodynamic septic shock in pigs has shown valuable effects in oxygen transport. Our goal was to assess the effects of levosimendan in a normodynamic model of endotoxaemia.
Methods: Twelve sheep were anaesthetized and mechanically ventilated. After taking basal haemodynamic and oxygen transport measurements, sheep were assigned to two groups during 120 min: (1) endotoxin (5 μg/kg endotoxin); (2) levosimendan (5 μg/kg endotoxin plus levosimendan 200 μg/kg followed by 200 μg/kg/h). Both groups received hydration of 20 ml/kg/h of saline solution.
Results: In the endotoxin group, cardiac output, intestinal blood flow and systemic and intestinal oxygen transports and consumptions (DO_2 and VO_2) remained unchanged. In the levosimendan group, systemic and intestinal DO_2 were significantly higher than in the endotoxin group. Because stroke volume did not change (basal versus 120′: 0.9 ± 0.1 ml/kg versus 0.9 ± 0.2 ml/kg, $p = 0.3749$), the elevation in cardiac output by levosimendan (145 ± 17 ml/min/kg versus 198 ± 16 ml/min/kg, $p = 0.0096$) was related to an increased heart rate (159 ± 32 beats l/min versus 216 ± 19 beats l/min, $p = 0.0037$). Levosimendan precluded the development of gut

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* Corresponding author. Tel.: +54 221 4220507; fax: +54 221 4790742.
E-mail address: arnaldodubin@speedy.com.ar (A. Dubin).

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intramucosal acidosis at 120′ (endotoxin versus levosimendan, ileal intramucosal-arterial P_{CO_2} difference: 19 ± 4 Torr versus 10 ± 4 Torr, $p = 0.0025$). However, levosimendan decreased mean arterial blood pressure (99 ± 20 Torr versus 63 ± 13 Torr, $p = 0.0235$) and increased blood lactate levels (2.4 ± 0.9 mmol/l versus 4.8 ± 1.5 mmol/l, $p = 0.0479$). All p-values are differences in specific points (paired or unpaired t-test with Bonferroni correction) after two-way repeated measures ANOVA. A p-value < 0.05 was considered significant.

Conclusions: Levosimendan improved oxygen transport and prevented the development of intramucosal acidosis in this experimental model of endotoxaemia. However, systemic hypotension and lactic acidosis occurred. Additional studies are needed to show if different doses and timing of levosimendan administration in septic shock might improve gut perfusion without adverse effects.

Introduction

Severe sepsis and septic shock are increasing health problems.[1] The pathophysiology of the haemodynamic derangement is complex and involves both cardiac and peripheral circulation dysfunction.[2] Fluid resuscitation is the cornerstone of haemodynamic management of septic shock.[3] However, evidence of tissue hypoperfusion frequently remains after adequate volume replacement,[4] so usually vasoactive drugs are required. Though, haemodynamics might be apparently corrected, hidden tissue dysoxia may persist. For example, adrenergic drugs might stabilize arterial blood pressure and cardiac output but might impair gut perfusion, and so fail to correct intramucosal acidosis.[5–7] Therefore, the search of drugs that might help during resuscitation of septic shock remains an important challenge. Recently, a new strategy has been tried for this purpose: to recruit the microcirculation by the use of vasodilators.[8]

Levosimendan, a new calcium-sensitizing inotropic drug, has improved cardiac function and survival in patients with congestive heart failure.[9] Levosimendan is also a vasodilator by stimulation of adenosine triphosphate (ATP)-sensitive potassium channel in vascular smooth muscle cells.[10] A preliminary report in hypodynamic experimental endotoxemic shock showed that levosimendan improved systemic and intestinal oxygen transport.[11]

We performed this experimental study (1) to assess the effects of levosimendan in haemodynamics and oxygen transport in a normodynamic model of endotoxaemia and (2) to test the hypothesis that vasodilatory effects of levosimendan might improve intramucosal acidosis, that is an increase of intramucosal-arterial P_{CO_2} difference (ΔP_{CO_2}), a key marker of tissue hypoperfusion in septic shock.

Materials and methods

Surgical preparation

Twelve sheep were anaesthetized with 30 mg/kg of sodium pentobarbital, intubated and mechanically ventilated (Harvard Apparatus Dual Phase Control Respirator Pump Ventilator, South Natick, MA, USA) with a tidal volume of 15 ml/kg, a F_IO_2 of 0.21 and PEEP adjusted to maintain O_2 arterial saturation >90%. The respiratory rate was set to keep the end-tidal P_{CO_2} at 35 mmHg. Neuromuscular blockade was performed with intravenous pancuronium bromide (0.06 mg/kg). Additional pentobarbital boluses (1 mg/kg/h) were administered as required.

Catheters were advanced through the left femoral vein to administer fluids and drugs, and through the left femoral artery to measure blood pressure and to obtain blood gases. A pulmonary artery catheter was inserted through the right external jugular vein (flow-directed thermodilution fiberoptic pulmonary artery catheter, Abbott Critical Care Systems, Mountain View, CA, USA).

An orogastric tube was inserted to allow drainage of gastric contents. Then, a midline laparotomy and splenectomy were performed.

An electromagnetic flow probe was placed around the superior mesenteric artery to measure intestinal blood flow. A catheter was placed in the mesenteric vein through a small vein proximal to the gut to draw blood gases. A tonometer was inserted through a small ileotomy to measure intramucosal P_{CO_2}. Lastly, after careful haemostasis, the abdominal wall incision was closed.

Measurements and derived calculations

Arterial, systemic, pulmonary and central venous pressures were measured with corresponding transducers (Statham P23 AA, Statham, Hato Rey, Puerto

Rico). Cardiac output was measured by thermodilution with 5 ml of 0 °C saline solution (HP OmniCare model 24 A 10, Hewlett-Packard, Andover, MA, USA). An average of three measurements taken randomly during the respiratory cycle was considered and was normalized to body weight (Q). Intestinal blood flow was measured by the electromagnetic method (Spectramed Blood Flowmeter model SP 2202 B, Spectramed Inc., Oxnard, CA, USA) with in vitro calibrated transducers of 5—7 mm of diameter (Blood Flowmeter Transducer, Spectramed Inc., Oxnard, CA, USA). Occlusive zero was controlled before and after each experiment. Non-occlusive zero was corrected before each measurement. Superior mesenteric blood flow was normalized to gut weight ($Q_{intestinal}$).

Arterial, mixed venous and mesenteric venous P_{O_2}, P_{CO_2} and pH were measured with a blood gas analyzer (ABL 5, Radiometer, Copenhagen, Denmark), and haemoglobin and oxygen saturation were measured with a cooximeter calibrated for sheep blood (OSM 3, Radiometer, Copenhagen, Denmark). Arterial, mixed venous and mesenteric venous contents (C_aO_2, C_vO_2 and $C_{vm}O_2$, respectively) were calculated as: $Hb \times 1.34 \times O_2$ saturation $+ P_{O_2} \times 0.0031$. Systemic and intestinal DO_2 and VO_2 (DO_2, VO_2, DO_{2i} and VO_{2i}, respectively) were calculated as $DO_2 = Q \times C_aO_2$; $VO_2 = Q \times (C_aO_2 - C_vO_2)$; $DO_{2i} = Q_{intestinal} \times C_aO_2$ and $VO_{2i} = Q_{intestinal} \times (C_aO_2 - C_{vm}O_2)$.

Intramucosal P_{CO_2} was measured with a tonometer (TRIP Sigmoid Catheter, Tonometrics Inc., Worcester, MA, USA) filled with 2.5 ml of saline solution. One milliliter was discarded after an equilibration period of 30 min and the P_{CO_2} was measured in the remaining 1.5 ml. Its value was corrected to the corresponding equilibration period and was used to calculate $\triangle P_{CO_2}$.

Mixed venous-arterial and mesenteric venous-arterial P_{CO_2} differences were also calculated. Arterial, mixed venous and mesenteric venous CO_2 contents (CCO_2) and their differences were calculated using Giovannini's algorithm.[12] Systemic and intestinal CO_2 production (VCO_2 and VCO_{2i}, respectively) were calculated as: $VCO_2 = CI \times$ mixed venoarterial CCO_2 and $VCO_{2i} = Q_{intestinal} \times$ mesenteric venoarterial CCO_2 difference.

Lactate was measured with an automatic analyzer (Automatic Analyzer Hitachi 912, Boehringer Mannheim Corporation, Indianapolis, IN, USA).

Experimental procedure

Basal measurements were taken after a stabilization period of no less than 30 min. Then, the F_IO_2 was increased to 0.50 and animals were assigned to two groups and treated as follows: (1) endotoxin group: 5 μg/kg of *Escherichia coli* lipopolysaccharide dissolved in 100 cm^3 of saline solution in 10 min, followed by saline solution; (2) levosimendan group: the same dose of endotoxin was administered, followed by a loading dose of the drug (Levosimendan; Orion Pharma, Espoo, Finland) (200 μg/kg in 10 min) and a continuous infusion (200 μg/kg/h) throughout the rest of the experiment. This dose is similar to that previously reported in an experimental study.[11]

Both groups were infused with the same volume of saline solution (20 ml/kg/h). This volume was chosen because in pilot experiments it maintained blood flow at basal levels after 5 μg/kg of endotoxin administration.

Measurements were performed at 30-min intervals during 120 min from the start of endotoxin administration.

Blood temperature was kept constant throughout the study with a heating lamp.

At the end of the experiment, the animals were killed with an additional dose of pentobarbital and a KCl bolus. A catheter was inserted in the superior mesenteric artery and Indian ink was instilled through it. Dyed intestinal segments were dissected, washed and weighted so as to calculate gut indexes.

This study was approved by the local Animal Care Committee. Care of animals was in accordance with National Institute of Health guidelines.

Statistical analysis

Data were assessed for normality and expressed as mean ± standard deviation (S.D.). They were analyzed with two-way repeated measures ANOVA. After an overall p-value for a between group comparison <0.05, differences in specific points of time were explored with unpaired t-test with Bonferroni correction for multiple comparisons. Within group differences were identified with paired t-test with Bonferroni. The software GraphPad PRISM version 3.02 was used.

Results

Haemodynamic and oxygen transport effects

The endotoxin group had no changes in systemic and intestinal blood flow and oxygen transport and consumption (Figure 1). Arterial blood pressure remained unchanged, but the pulmonary

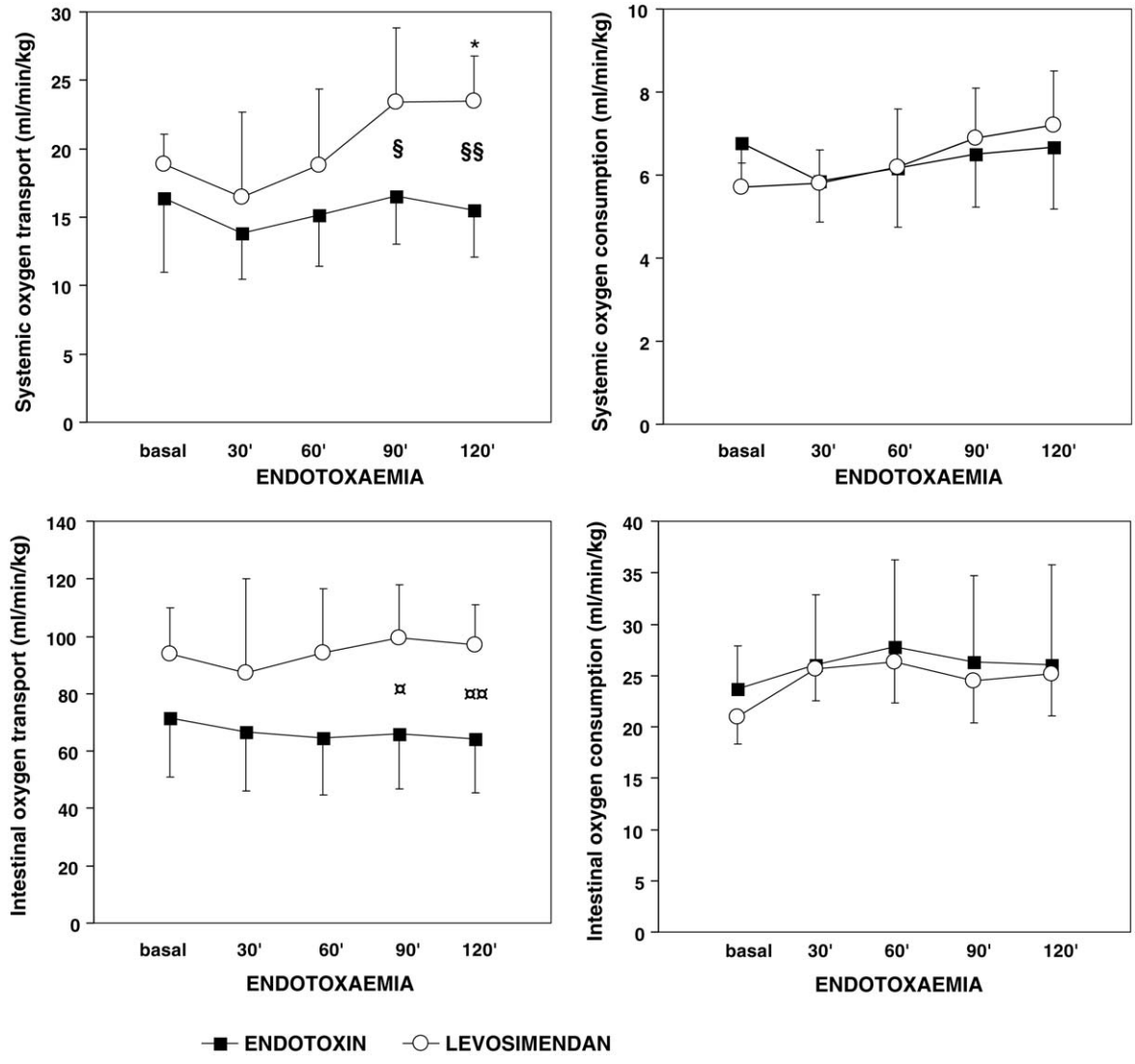

Figure 1 Systemic and intestinal oxygen transport and consumption in the endotoxin and levosimendan groups. Systemic and intestinal oxygen transport and consumption remained stable in endotoxin group. Levosimendan increased systemic and intestinal oxygen transport while systemic and intestinal oxygen consumption stayed unchanged. $^{\S}p = 0.0476$ at 90′ and $^{\S\S}p = 0.0107$ at 120′ for endotoxin vs. levosimendan groups (unpaired *t*-test with Bonferroni correction after two-way repeated measures ANOVA = 0.0014). $^{*}p = 0.0481$ for basal vs. 120′, in levosimendan group (paired *t*-test with Bonferroni correction). $^{¤}p = 0.0489$ at 90′ and $^{¤¤}p = 0.0413$ at 120′ for endotoxin vs. levosimendan groups (unpaired *t*-test with Bonferroni correction after two-way repeated measures ANOVA = 0.0004).

artery pressure and vascular resistance were elevated (Table 1). The levosimendan group increased systemic and intestinal blood flow and oxygen transport without changes in oxygen consumption (Figure 1). In addition, systemic hypotension and reduction of pulmonary vascular resistance occurred (Table 1).

Effects on intramucosal acidosis and CO_2 derived variables

ΔP_{CO_2} increased in the endotoxin group and remained unchanged in the levosimendan group. Mesenteric venous-arterial P_{CO_2} was also lower in the levosimendan group (Table 2 and Figure 2). There were no statistically significant differences in systemic and intestinal CO_2 production between groups (Table 2).

Effects on lactate levels and acid—base metabolism

Blood lactate levels slightly increased in the endotoxin group and were raised markedly in the levosimendan group (Figure 3).

Table 1 Systemic and intestinal hemodynamic variables in basal conditions and after endotoxin administration in the endotoxin (ENDO) and the levosimendan (LEVO) groups

	Group	Basal	Endotoxemia				*p*-value[#]
			30 min	60 min	90 min	120 min	
Heart rate (beats/min)	ENDO	152 ± 34	153 ± 40	147 ± 37	164 ± 33	163 ± 21	0.0039
	LEVO	159 ± 32	175 ± 62	190 ± 43[*]	215 ± 28[*,§]	216 ± 19[*,§]	
Mean arterial pressure (mmHg)	ENDO	92 ± 20	94 ± 11	88 ± 19	97 ± 26	99 ± 20	<0.0001
	LEVO	92 ± 13	62 ± 25[*,§]	53 ± 16[*,§]	58 ± 13[*,§]	63 ± 13[*,§]	
Mean pulmonary arterial pressure (mmHg)	ENDO	15 ± 5	34 ± 6[*]	27 ± 7[*]	26 ± 5[*]	26 ± 6[*]	0.0729
	LEVO	18 ± 3	32 ± 8[*]	21 ± 4	21 ± 3	22 ± 4[*]	
Pulmonary artery wedge pressure (mmHg)	ENDO	5 ± 2	10 ± 4[*]	7 ± 3[*]	8 ± 3[*]	8 ± 5	0.7942
	LEVO	6 ± 3	8 ± 4	8 ± 3	8 ± 2	8 ± 3	
Central venous pressure (mmHg)	ENDO	4 ± 2	4 ± 3	5 ± 2	5 ± 1	5 ± 2	0.6975
	LEVO	2 ± 1	5 ± 2[*]	5 ± 3[*]	5 ± 2[*]	4 ± 1[*]	
Cardiac output (ml/min/kg)	ENDO	145 ± 46	122 ± 16	148 ± 31	158 ± 37	146 ± 37	0.0120
	LEVO	145 ± 17	131 ± 29	157 ± 29	201 ± 34[*]	198 ± 16[*,§]	
Stroke volume (ml/kg)	ENDO	1.0 ± 0.4	0.8 ± 0.2	1.0 ± 0.3	1.0 ± 0.2	0.9 ± 0.2	0.3749
	LEVO	0.9 ± 0.2	0.8 ± 0.2	0.9 ± 0.2	1.0 ± 0.3	0.9 ± 0.1	
Superior mesenteric artery blood flow (ml/min/kg)	ENDO	637 ± 192	598 ± 152	635 ± 175	626 ± 157	601 ± 163	0.0039
	LEVO	712 ± 94	685 ± 133	791 ± 118	853 ± 128[*,§]	816 ± 98[*,§]	
Systemic vascular resistance (dynes s/cm^5)	ENDO	2657 ± 1106	2911 ± 747	2305 ± 793	2430 ± 947	2653 ± 710	0.0011
	LEVO	2880 ± 438	1949 ± 528[*]	1400 ± 292[*,§]	1271 ± 316[*,§]	1366 ± 147[*,§]	
Pulmonary vascular resistance (dynes s/cm^5)	ENDO	266 ± 89	740 ± 103[*]	569 ± 269[*]	477 ± 190[*]	505 ± 147[*]	0.0456
	LEVO	374 ± 148	870 ± 222[*]	390 ± 147	293 ± 108	330 ± 108[§]	

[#] Overall *p*-value for comparisons between groups (two-way repeated measures ANOVA).
[*] $p < 0.05$ vs. basal (paired *t*-test with Bonferroni correction).
[§] $p < 0.05$ vs. ENDO (unpaired *t*-test with Bonferroni correction).

Arterial, mixed venous and mesenteric venous blood gases are shown in Table 3. There was a non-statistically significant tendency to a higher degree of metabolic acidosis in the levosimendan group.

Effects on pulmonary oxygenation

PaO_2/F_IO_2 showed a similar reduction in both groups (421 ± 95 versus 267 ± 96 and 376 ± 57 versus 294 ± 98, $p < 0.05$ versus basal for both groups).

Discussion

This study confirms previous findings related to levosimendan-induced increase in oxygen transport in experimental endotoxaemic shock.[11] It also shows that increases in blood flow and oxygen transport were associated with correction of intramucosal acidosis in normodynamic endotoxaemia. Another key finding was the marked elevation of blood lactate levels associated with a high rate of levosimendan infusion, implying that excessive vasodilation might be harmful to tissue oxygenation.

The experimental model of septic shock

Bolus administration or short-term infusion of endotoxin usually induces a low flow state followed by vasoconstriction.[13] Long-term infusion of endotoxin results in a hyperdynamic state with a low systemic vascular resistance.[14] We used a short-term endotoxin infusion followed by expansion with saline to produce a normodynamic shock pattern, with preserved cardiac output and superior mesen-

Table 2 Systemic and intestinal CO_2 variables in basal conditions and after endotoxin administration in the endotoxin (ENDO) and the levosimendan (LEVO) groups

	Group	Basal	Endotoxaemia				*p*-value[#]
			30 min	60 min	90 min	120 min	
Mixed venous-arterial P_{CO_2} (mmHg)	ENDO	8 ± 2	8 ± 2	7 ± 2	8 ± 3	9 ± 2	0.3140
	LEVO	6 ± 2	7 ± 3	7 ± 5	8 ± 3	7 ± 2	
Mesenteric venous-arterial P_{CO_2} (mmHg)	ENDO	8 ± 2	8 ± 2	10 ± 3	11 ± 4	11 ± 2	0.0017
	LEVO	6 ± 2	7 ± 5	7 ± 3	8 ± 5	7 ± 2[§]	
Intramucosal-arterial P_{CO_2} (mmHg)	ENDO	7 ± 4	6 ± 5	12 ± 5	15 ± 6[*]	19 ± 4[*]	0.0025
	LEVO	4 ± 6	1 ± 5	10 ± 9	12 ± 4	10 ± 2[§]	
Systemic CO_2 production (ml/min/kg)	ENDO	6.8 ± 2.6	4.7 ± 1.5	5.7 ± 1.6	5.8 ± 1.2	5.5 ± 1.9	0.5289
	LEVO	5.8 ± 1.7	4.5 ± 1.9	4.8 ± 2.4	7.8 ± 3.7	7.1 ± 2.1	
Intestinal CO_2 production (ml/min/kg)	ENDO	27.9 ± 8.8	24.0 ± 9.7	26.0 ± 8.4	30.6 ± 17.3	27.6 ± 10.8	1.0000
	LEVO	21.0 ± 9.5	25.0 ± 7.8	23.6 ± 12.3	37.6 ± 20.0	28.8 ± 11.0	

[#] Overall *p*-value for comparisons between groups (two-way repeated measures ANOVA).
[*] $p < 0.05$ vs. basal (paired *t*-test with Bonferroni correction).
[§] $p < 0.05$ vs. ENDO (unpaired *t*-test with Bonferroni correction).

teric artery blood flow. We chose a normodynamic model of shock in order to avoid macrovascular hypoperfusion-mediated CO_2 accumulation. However, despite maintenance of systemic and gut oxy-

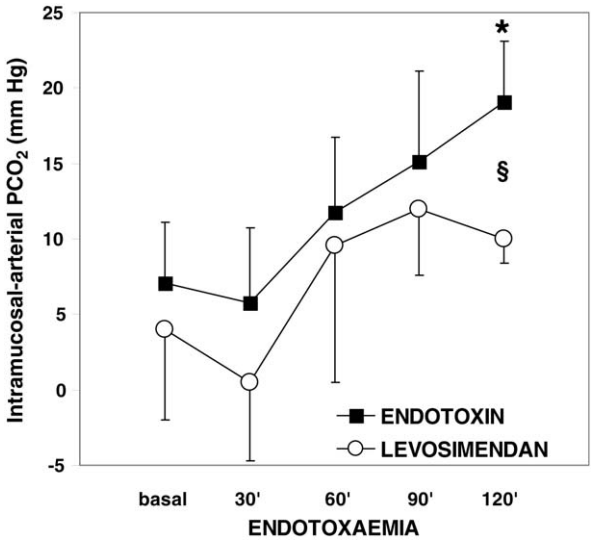

Figure 2 Intramucosal-arterial P_{CO_2} difference (ΔP_{CO_2}) in the endotoxin and levosimendan groups. ΔP_{CO_2} increased after endotoxin injection. Levosimendan prevented the development of intramucosal acidosis. [§]$p = 0.0025$ at 120′ for endotoxin vs. levosimendan groups (unpaired *t*-test with Bonferroni correction after two-way repeated measures ANOVA = 0.0025). [*]$p = 0.0261$ for basal vs. 120′ (paired *t*-test with Bonferroni correction).

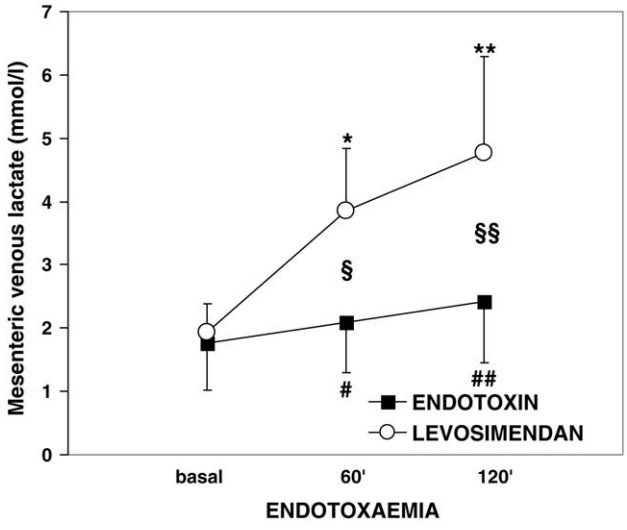

Figure 3 Mesenteric venous lactate in endotoxin and levosimendan groups. Blood lactate levels slightly increased in endotoxin group and markedly raised in levosimendan group. [§]$p = 0.0290$ at 60′ and [§§]$p = 0.0287$ at 120′ for endotoxin vs. levosimendan groups (unpaired *t*-test with Bonferroni correction after two-way repeated measures ANOVA = 0.0016). [*]$p = 0.0219$ and [**]$p = 0.0137$ for basal vs. 60′ and 120′, respectively, in the levosimendan group (paired *t*-test with Bonferroni correction). [#]$p = 0.0489$ and [##]$p = 0.0413$ for basal vs. 60′ and 120′, respectively, in the endotoxin group (paired *t*-test with Bonferroni correction).

Table 3 Arterial, mixed venous and mesenteric venous gases in basal conditions and after endotoxin administration in the endotoxin (ENDO) and the levosimendan (LEVO) groups

	Group	Basal	Endotoxemia				p-value[#]
			30 min	60 min	90 min	120 min	
Arterial pH	ENDO	7.38 ± 0.07	7.33 ± 0.09	7.30 ± 0.05	7.33 ± 0.05	7.34 ± 0.07	0.3412
	LEVO	7.43 ± 0.05	7.28 ± 0.06*	7.28 ± 0.09*	7.29 ± 0.08*	7.29 ± 0.08*	
Arterial P_{CO_2} (Torr)	ENDO	36 ± 4	40 ± 7	42 ± 8	38 ± 6	37 ± 7	0.1647
	LEVO	39 ± 6	46 ± 6*	44 ± 9	42 ± 9	38 ± 9	
Arterial P_{O_2} (Torr)	ENDO	88 ± 19	126 ± 61	103 ± 32	132 ± 16*	133 ± 48*	0.7476
	LEVO	79 ± 12	102 ± 33	126 ± 51*	147 ± 41*	147 ± 49*	
Arterial HCO_3 (mmol/l)	ENDO	21 ± 3	21 ± 3	20 ± 3	20 ± 3	20 ± 3	0.4508
	LEVO	26 ± 5	21 ± 3	20 ± 3*	20 ± 3*	18 ± 3*	
Arterial base excess (mmol/l)	ENDO	−3 ± 4	−5 ± 4	−6 ± 4*	−5 ± 3*	−5 ± 3*	0.9330
	LEVO	2 ± 5	−5 ± 4*	−8 ± 4*	−6 ± 4*	−8 ± 3*	
Mixed venous pH	ENDO	7.34 ± 0.07	7.29 ± 0.08*	7.27 ± 0.05*	7.28 ± 0.06*	7.28 ± 0.07*	0.6158
	LEVO	7.39 ± 0.04	7.25 ± 0.06*	7.24 ± 0.08*	7.25 ± 0.08*	7.25 ± 0.08*	
Mixed venous P_{CO_2} (Torr)	ENDO	43 ± 5	47 ± 8	49 ± 9	46 ± 7	45 ± 9	0.3494
	LEVO	45 ± 7	53 ± 7*	52 ± 9	50 ± 11	45 ± 9	
Mixed venous P_{O_2} (Torr)	ENDO	36 ± 6	39 ± 10	41 ± 8	40 ± 6	39 ± 7	0.1946
	LEVO	39 ± 2	41 ± 7	44 ± 4*	47 ± 4*,§	47 ± 3*	
Mixed venous HCO_3 (mmol/l)	ENDO	23 ± 2	22 ± 2	22 ± 3	21 ± 2*	21 ± 3*	0.4679
	LEVO	27 ± 5	23 ± 3	22 ± 3*	22 ± 4*	20 ± 3*	
Mixed venous base excess (mmol/l)	ENDO	−2 ± 4	−4 ± 3*	−5 ± 3*	−5 ± 3*	−5 ± 3*	0.8372
	LEVO	2 ± 5	−5 ± 3*	−6 ± 4*	−5 ± 4*	−7 ± 4*	
Mesenteric venous pH	ENDO	7.34 ± 0.07	7.28 ± 0.09	7.24 ± 0.06*	7.26 ± 0.06*	7.27 ± 0.07*	0.8695
	LEVO	7.40 ± 0.04	7.26 ± 0.07*	7.25 ± 0.08*	7.26 ± 0.08*	7.26 ± 0.08*	
Mesenteric venous P_{CO_2} (Torr)	ENDO	43 ± 5	48 ± 8	52 ± 9	49 ± 8	47 ± 41	0.8367
	LEVO	44 ± 7	53 ± 8*	51 ± 9	50 ± 12	45 ± 9	
Mesenteric venous P_{O_2} (Torr)	ENDO	39 ± 5	39 ± 10	38 ± 5	41 ± 4	41 ± 8	0.0003
	LEVO	43 ± 3	44 ± 8	47 ± 4§	52 ± 7*,§	50 ± 6*,§	
Mesenteric venous HCO_3 (mmol/l)	ENDO	23 ± 2	22 ± 2	22 ± 2	22 ± 3*	21 ± 3*	0.5547
	LEVO	27 ± 5	23 ± 3	22 ± 3*	22 ± 5*	20 ± 3*	
Mesenteric venous base excess (mmol/l)	ENDO	−2 ± 3	−4 ± 4*	−5 ± 3*	−5 ± 3*	−5 ± 3*	0.6845
	LEVO	2 ± 5	−4 ± 4*	−6 ± 4*	−5 ± 5*	−7 ± 4*	

[#] Overall p-value for comparisons between groups (two-way repeated measures ANOVA).
* $p < 0.05$ vs. basal (paired t-test with Bonferroni correction).
§ $p < 0.05$ vs. ENDO (unpaired t-test with Bonferroni correction).

gen transport and consumption, intramucosal acidosis developed.

The reason for increased intestinal ΔP_{CO_2} in sepsis remains controversial. In some experimental models, increased ΔP_{CO_2} reflects low blood flow.[15] In contrast, other investigators have described the occurrence of intramucosal acidosis in normodynamic models. Vallet et al. studied endotoxaemic dogs with low blood flow resuscitated with dextran. Gut flow was increased and oxygen transport normalized. Nevertheless, oxygen uptake and mucosal P_{O_2} and pH remained low.[16] The authors

speculated about flow redistribution from mucosal to serosal layers. However, Revelly et al. described an endotoxin-induced redistribution from serosa to mucosa and an inverse correlation between intramucosal blood flow and pH.[17] VanderMeer et al. found that intramucosal acidosis developed despite preserved blood flow and tissue P_{O_2}, in endotoxaemic pigs.[18] They hypothesized about the presence of metabolic derangement as the underlying mechanism. Further research from the same group described some of these alterations as associated to the PARD complex, and introduced the term of cytopathic hypoxia.[19] However, tissue hypoxia and increased anaerobic CO_2 production might not be the only explanation for ΔP_{CO_2} rise. Vallet et al. [20] and Dubin et al. [21,22] recently showed that hypoperfusion is needed for the development of intramucosal acidosis. In addition, Tugtekin et al. demonstrated the correlation between increased ΔP_{CO_2} and diminished villi microcirculation, despite the preservation of global gut blood flow.[23] Moreover, supranormal elevation of blood flow prevents intramucosal acidosis in sheep endotoxaemia.[24] This body of information allows concluding that intramucosal acidosis in sepsis is mainly due to microcirculatory derangements, even though the cardiac output and regional flow remain unchanged. Alterations in energy metabolism and cellular oxygen utilization could contribute to multiple organ failure development, but they do not explain the development of mucosal acidosis.

As previously described in sheep, lipopolysaccharide infusion caused acute lung injury.[25] The main pulmonary findings of endotoxin administration were pulmonary hypertension and impairment of pulmonary oxygenation.

The endotoxin group might be described as suffering from severe sepsis and tissue hypoperfusion, the so-called ''occult shock''.[4] An increased ΔP_{CO_2} was its main feature, which points to the usefulness of gut tonometry as an early and sensitive marker. Lactate levels were slightly but significantly elevated in mesenteric venous blood.

The cardiovascular response to levosimendan

Levosimendan administration resulted in a hyperdynamic state with high cardiac output, tachycardia, and systemic and pulmonary vasodilation.

Unexpectedly, the increase in cardiac output was primarily due to tachycardia. Levosimendan effects on cardiac output are due to improved contractility, and, additionally, to systemic and pulmonary vasodilation. In a previous study in pigs, levosimendan was unable to return cardiac output to basal levels after endotoxin administration, but, still, cardiac output was higher than in controls with endotoxaemia only. Stroke volume during endotoxemia was reduced in levosimendan group, but not different from controls.[11] In our experiments, the stroke volume remained unchanged in both groups. The reasons for the lack of contractility improvement are unknown. In a study in endotoxin-exposed guinea pigs, levosimendan failed to reverse left ventricular dysfunction.[26] A possible explanation for these effects is that the acidosis that developed in our experiment might have blunted levosimendan inotropic effect.[27]

As previously reported, important vasodilatory effects were noticed. Vasodilation was more evident at systemic level, causing reductions in mean arterial blood pressure and systemic vascular resistance. This could be an undesired effect in clinical settings, due to the frequent presence of hypotension in sepsis. Pulmonary arterial pressures showed a tendency to be lower and pulmonary vascular resistance was clearly reduced in levosimendan group. This effect could also contribute to improve cardiac output.

Oxygen transport and tissue oxygenation responses to levosimendan

Levosimendan increased systemic and intestinal oxygen transport but systemic and intestinal oxygen consumption remained unchanged. Despite the lack of oxygen supply dependency, levosimendan-induced increases in oxygen transport were associated with prevention of intramucosal acidosis. This effect was evident at the end of the experiments, possible due to a time related effect of endotoxin and levosimendan. ΔP_{CO_2} is the result of interaction between CO_2 production, the capacity of the blood to transport CO_2 and blood flow to tissues. Since the only difference in these variables between both groups was blood flow, our data suggest that intramucosal acidosis in the endotoxin control group was mainly determined by local hypoperfusion. On the other hand, vasodilation produced by levosimendan could correct this perfusion defect. In a recently published study,[11] levosimendan failed to correct intramucosal acidosis. However, in that study, the intestinal blood flow remained lower than the level before endotoxin administration. Consequently, differences between both studies might be related to the level of blood flow achieved.

Inadequate mucosal perfusion could be a relevant event in critically illness, by inducing damage

to, and breakdown of, the mucosal barrier, further translocation of bacteria or their byproducts to systemic circulation, and initiation of events leading to multiple organ failure.[28,29] Recently suggested strategies to improve tissue oxygenation in septic shock have pointed to opening the microcirculation and holding it open.[8] Clinical studies have shown that nitrovasodilators might correct microcirculatory defects [30,31] and our results suggest that the vasodilatory effects of levosimendan could also serve this purpose.

Effects on lactate levels

The elevation of lactate in levosimendan group was another relevant finding of this study. Hyperlactatemia warns of the development of tissue dysoxia in vascular beds other than the gut. Different mechanisms could explain these findings: levosimendan-induced vasodilation might produce blood flow diversion with mismatch between oxygen flux and metabolic needs. Some tissues could so become dysoxic because of a steal phenomenon. In addition, systemic hypotension might trigger an adrenergic response with tachycardia, increased cardiac work and a calorigenic effect, producing a state of increased metabolic needs. Total oxygen consumption might not change as consequence of opposing behaviour in regional uptakes. In this way, raised lactate levels might be considered possible markers of tissue dysoxia, a state in which DO_2 can no longer sustain VO_2 and aerobic metabolism.[32] Since there is no gold standard to track tissue oxygenation, a comprehensive clinical approach should assess different variables, as considered in this experimental study.

Another explanation for hyperlactataemia might reside in the aerobic production of lactate. We are not aware of any studies describing levosimendan effects on carbohydrate metabolism. However, we can speculate about the effects of endogenously released epinephrine in response to hypotension. Physiologically, epinephrine (adrenaline) enhances glycogenolysis with a net increase in pyruvate production. This mechanism is related to a β_2-adrenergic-mediated stimulation of muscle and hepatic phosphorylase, and inhibition of glycogen synthetase.[33,34] Recently, Levy et al. have shown that epinephrine-induced hyperlactatemia in endotoxaemic rodents is probably related to direct effects on carbohydrate metabolism and not to cellular hypoxia.[35]

Due to levosimendan-induced vasodilation, relative hypovolemia might be present. However, central venous and pulmonary wedge pressures did not decrease in either group. Notwithstanding this, more aggressive resuscitation could have diminished lactic acidosis.

Whatever the cause, the rise in blood lactate warns about the clinical use of levosimendan before more basic research is done.

Effects of levosimendan on pulmonary oxygenation

Elevation in cardiac output or administration of pulmonary vasodilators increase intrapulmonary shunt.[36] Despite having both effects, levosimendan produced non-significant changes in arterial P_{O_2} or in PaO_2/F_IO_2. This was expected, as has been previously discussed,[37] due to the opposite effects of increased shunt and mixed venous P_{O_2} on arterial P_{O_2}. In addition, the lack of impairment in oxygenation might be related to the pathogenesis of lung injury after endotoxin injection, in which proinflammatory mechanisms and a hydrostatic component are involved.[38] Levosimendan vasodilatory effects might diminish capillary pressure and so decrease oedema formation, offsetting oxygenation changes due to haemodynamic causes.

Conclusions

In this experimental model of septic shock, levosimendan improved oxygen transport and prevented the development of intramucosal acidosis. However, systemic hypotension and lactic acidosis arose. These results do not address the issue of levosimendan use in human septic shock but warn of potential detrimental effects. Additional studies are needed to show if different doses and timing of levosimendan administration in septic shock might improve gut perfusion without adverse effects.

Conflict of interest statement

The authors declare no conflict of interest.

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