ACTA CIRÚRGICA BRASILEIRA

ORIGINAL ARTICLE
Experimental Surgery
https://doi.org/10.1590/acb370703

Effects of dexmedetomidine on hemodynamic, oxygenation, microcirculation, and inflammatory markers in a porcine model of sepsis

Paulo Carnicelli[1], Denise Aya Otsuki[2]*, Adalberto Monteiro Filho[3], Marcia Aparecida Portela Kahvegian[4], Keila Kazue Ida[5], José Otavio Costa Auler-Jr.[6], Jean-Jacques Rouby[7], Denise Tabacchi Fantoni[8]

1. MSc. Universidade de São Paulo – Faculdade de Medicina Veterinária e Zootecnia – Surgery Department – São Paulo (SP), Brazil.
2. PhD. Universidade de São Paulo – Hospital das Clínicas da Faculdade de Medicina – LIM08-Laboratory of Anesthesiology – São Paulo (SP), Brazil.
3. MSc. Clínica Ufape – Veterinary Intensive Care Unit – Garanhuns (PE), Brazil.
4. PhD. All Care Vet – Sao Paulo (SP), Brazil.
5. PhD. Texas A&M University – College of Veterinary Medicine and Biomedical Sciences – Department of Small Animal Clinical Sciences – College Station (TX), United States of America.
6. PhD. Universidade de São Paulo – Hospital das Clínicas da Faculdade de Medicina – Laboratory of Anesthesiology – São Paulo (SP), Brazil.
7. PhD. Assistance Publique Hôpitaux de Paris – La Pitié Salpêtrière Hospital – Multidisciplinary Intensive Care Unit – Medicine Sorbonne University – Department of Anaesthesiology and Critical Care – Paris, France.
8. PhD. Universidade de São Paulo – Faculdade de Medicina Veterinária e Zootecnia – Surgery Department – São Paulo (SP), Brazil.

ABSTRACT

Purpose: To determine whether dexmedetomidine aggravates hemodynamic, metabolic variables, inflammatory markers, and microcirculation in experimental septic shock. **Methods:** Twenty-four pigs randomized into: Sham group (n = 8), received saline; Shock group (n = 8), received an intravenous infusion of *Escherichia coli* O55 (3×10^9 cells/mL, 0.75 mL/kg, 1 hour); Dex-Shock group (n = 8), received bacteria and intravenous dexmedetomidine (bolus 0.5 mcg/kg followed by 0.7 mcg/kg/h). Fluid therapy and/or norepinephrine were administered to maintain a mean arterial pressure > 65 mmHg. Hemodynamic, metabolic, oxygenation, inflammatory markers, and microcirculation were assessed at baseline, at the end of bacterial infusion, and after 60, 120, 180, and 240 minutes. **Results:** Compared to Shock group, Dex-Shock group presented a significantly increased oxygen extraction ratio at T180 (23.1 ± 9.7 vs. 32.5 ± 9.2%, P = 0.0220), decreased central venous pressure at T120 (11.6 ± 1 vs. 9.61 ± 1.2 mmHg, P = 0.0214), mixed-venous oxygen saturation at T180 (72.9 ± 9.6 vs. 63.5 ± 9.2%, P = 0.026), and increased plasma lactate (3.7 ± 0.5 vs. 5.5 ± 1 mmol/L, P = 0.003). Despite the Dex-Shock group having a better sublingual vessel density at T240 (12.5 ± 0.4 vs. 14.4 ± 0.3 mL/m^2; P = 0.0003), sublingual blood flow was not different from that in the Shock group (2.4 ± 0.2 vs. 2.4 ± 0.1 mL/kg, P = 0.4418). **Conclusions:** Dexmedetomidine did not worsen the hemodynamic, metabolic, inflammatory, or sublingual blood flow disorders resulting from septic shock. Despite inducing a better sublingual vessel density, dexmedetomidine initially and transitorily increased the mismatch between oxygen supply and demand.

Key words: Sepsis. Dexmedetomidine. Swine.

Introduction

Sepsis is frequently observed in critically ill patients and a common cause of mortality. It involves a massive release of inflammatory mediators in response to an injury caused by pathogenic microorganisms in different organs, and it is

*Corresponding author: denise.otsuki@hc.fm.usp.br | (55 11) 999603385
Received: Mar 12, 2022 | **Review:** May 9, 2022 | **Accepted:** June 13, 2022
Conflict of interest: Nothing to declare.
Research performed at Surgery Department, Faculdade de Medicina Veterinária e Zootecnia, and LIM08-Laboratory of Anesthesiology, Faculdade de Medicina, Universidade de São Paulo (USP), São Paulo (SP), Brazil.

clinically characterized by arterial hypotension, pulmonary hypertension, endothelial injury, and coagulation disorders. Persistent tissue hypoxia results from microcirculatory impairment and can be followed by the development of ischaemic reperfusion injury and multiple organ failure[1,2].

Patients in the intensive care unit (ICU) are often uncomfortable because of anxiety, pain, and mechanical ventilation. This discomfort can be treated with analgesics and sedatives, which also facilitate nursing care. Among the different classes of agents, dexmedetomidine is an α2-adrenoceptor agonist with a high affinity for α2-receptors (α2:α1 ratio of 1,620:1), a high potency, and fewer side effects are related to the activation of α1-receptors[3]. The mechanism of action is characterized by the activation of both pre- and postsynaptic α2-adrenoreceptors. Presynaptic activation inhibits the release of norepinephrine and, consequently, modulates pain signalling pathways. In the central nervous system, postsynaptic activation inhibits sympathetic tone, decreasing the heart rate, and blood pressure. The sympatholytic effect reduces the stress response and avoids changes in hemodynamic patterns caused by an increased release of endogenous catecholamines. These combined mechanisms inhibit neuronal firing, produce sedation and analgesia, and decrease nausea, salivation, secretion, and intestinal motility[4]. The onset of action of dexmedetomidine is observed approximately 15 minutes after the beginning of the infusion, reaching the maximum effect after 1 hour[3]. The synergistic effect with other analgesics reduces the requirement for opioids during surgery and in the postoperative period, decreasing the incidence of respiratory depression caused by opioids[3,4]. In addition, dexmedetomidine has important effects on the immune response, which mainly result from the central sympatholytic effects of dexmedetomidine and its binding to alpha-2 adrenoceptors in macrophages[5,6].

Despite the potential benefits of dexmedetomidine for critically ill patients, the drug can also be associated with adverse effects, including initial arterial hypertension followed by hypotension, bradycardia, atrial fibrillation, nausea, and hypoxia. At higher doses, first- and second-degree atrioventricular blockages can be observed[3,7].

Dexmedetomidine facilitates the clinical care of critically ill patients by improving their comfort and preventing delirium[8,9]. Given the dexmedetomidine-induced cardiovascular and respiratory depression, the benefit/disadvantage ratio for patients with septic shock is unknown. Therefore, the aim of this study was to assess whether dexmedetomidine worsens hemodynamic, oxygenation, metabolic, inflammatory, and microcirculatory responses in a model of septic shock. We hypothesised that dexmedetomidine would not further deteriorate sepsis disorders related disorders or would even improve microcirculatory conditions.

Methods

This prospective randomized experimental study was approved by the Ethics Committee for research projects at our institution (#1,420/2008). All animals received human care in compliance with the Principles of Laboratory Animal Care formulated by the National Society for Medical Research and the US National Academy of Sciences Guide for the Care and Use of Laboratory Animals.

Animal preparation

Twenty-four Landrace and Large White crossbred female pigs weighing 24.1 ± 2.4 kg were used in the study. The animals were fasted for 12 hours with free access to water before the experiments. Animals were premedicated with midazolam (0.25 mg/kg) and ketamine (5 mg/kg) intramuscularly. Anaesthesia was induced with propofol (5 mg/kg) administered intravenously (IV) and, after endotracheal intubation, maintained with isoflurane (1.4% end-tidal concentration) vaporized in 40% oxygen. Pancuronium was administrated (bolus of 0.1 mg/kg followed by infusion of 0.02 mg/kg/min), and mechanical ventilation (Primus; Dräger, Lübeck, Germany) was performed using the volume-controlled ventilation mode with a tidal volume of 8 mL/kg, a positive end-expiratory pressure (PEEP) of 5 cmH_2O and the respiratory rate adjusted to maintain an end-tidal carbon dioxide ($ETCO_2$) between 35-45 mmHg. Local anaesthesia was performed by administering 3 mL of 2% lidocaine at each incision site. Lactated Ringer's solution was administered at 5 mL/kg/h during preparation and at 10 mL/kg/h

during the experimentation period. Body temperature was maintained between 37-38 °C by using a heated mat (Medi-therm II; Gaymar Industries, Orchard Park, NY, United States of America).

Experimental protocol

Bacterial preparation

A strain of *Escherichia coli* (EPEC, O55) from VPS-FMVZ-USP was activated in trypticase soy broth (TSB) for 24 hours, spread on trypticase soy agar (TSA), and incubated for 24 hours at 37 °C. After bacterial growth, aliquots were suspended and diluted in saline to obtain a solution of 3×10^9 cells/mL, which corresponded to 0.6×10^{10} ufc/mL/live *E. coli*. The target concentration of bacteria was measured via spectrophotometry, with a final absorbance between 0.990 and 0.960[10]. The bacteria solution was stored at 4 °C for 12 to 36 hours prior to IV administration to the animals.

Experimental design

Following surgical preparation, baseline data were obtained, and animals were randomly allocated into one of the following three groups:

- a Shock group (n = 8) consisting of animals that received a 0.75-mL/kg infusion of *E. coli* O55 solution for 60 minutes[11];
- a Dex-Shock group (n = 8), that simultaneously received infusions of bacteria and dexmedetomidine (bolus of 0.5 µg/kg in 10 minutes, followed by a constant rate infusion of 0.7 µg/kg/h until the end of the experiment);
- a Sham group (n = 8), that did not receive the bacteria or dexmedetomidine infusion.

The Sham and Shock groups received saline solution at an infusion rate equivalent to that of dexmedetomidine. Randomization was previously performed, and the group allocation was blindly placed in numbered manila envelopes, which were opened in a consecutive manner immediately before baseline measurements were registered.

After sepsis induction, the animals were monitored and treated from T0 to T240. A bolus of 20 mL/kg lactated Ringer's solution was infused within 20 minutes if they had arterial hypotension (mean arterial pressure – MAP < 65 mmHg), central venous pressure (CVP) ≤ 12 mmHg, mixed-venous oxygen saturation (SvO_2) < 65% and urine output < 0.5 mL/kg/h. If these alterations were present with a CVP > 12 mmHg, the animals received a norepinephrine infusion (starting rate of 0.1 µg/kg/min, with the dose increasing by 0.05 µg/kg/min every 5 minutes, for up to 2 µg/kg/min) until hemodynamic stabilization was achieved[12]. The volume of additional fluids, norepinephrine requirements, and urine output were recorded (Fig. 1).

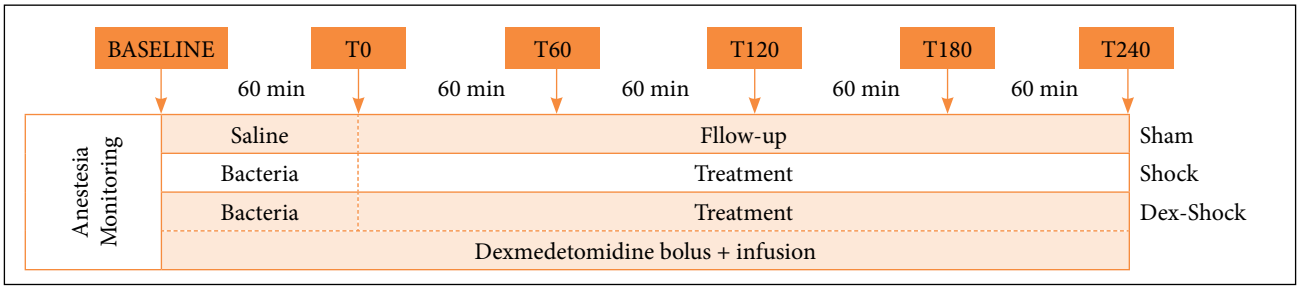


Figure 1 - Experimental design.

Measurements

Hemodynamics and blood gas analysis

Both the femoral artery and vein were catheterized for arterial pressure monitoring, blood sampling, and fluid administration. A 7.5-F pulmonary artery catheter (Swan-Ganz; Edwards Lifesciences, Irvine, CA, United States of America)

was surgically introduced into the right internal jugular vein and advanced under continuous pressure recording into wedge position. Cardiac output was determined by the thermodilution method (Vigilance monitor; Edwards Lifesciences). The cardiac index (CI) was calculated to normalize the data for body surface area in square meters by using a conversion factor appropriate for pigs (Eq. 1):

$$k \times BW^{2/3} \quad (1)$$

In which: $k = 0.09$; BW = body weight in kg[13].

The heart rate (HR), MAP, CVP, mean pulmonary artery pressure (MPAP), and pulmonary artery occlusion pressure (PAOP) were continuously monitored with a multiparametric monitor (IntelliVue MP50, Philips Healthcare, Best, Netherlands). The systemic vascular resistance index (SVRI), pulmonary vascular resistance index (PVRI), stroke volume index (SVI), systemic oxygen delivery index (DO_2I), systemic oxygen consumption index (VO_2I), and systemic oxygen extraction ratio (O_2ER) were calculated utilizing standard formulae. Arterial and mixed venous blood samples were collected simultaneously at each time point and immediately analysed (ABL 555; Radiometer, Copenhagen, Denmark) for blood gas analyses, including measurement of haemoglobin (Hb), lactate, and potassium (K^+). Blood glucose was assessed with a portable device (Accu-Check Advantage II; Roche, Mannheim, Germany). A 5%-glucose solution was used when necessary to maintain blood glucose > 40 mg/dL. The volume of 5% glucose solution administered, if any, was recorded.

Sublingual microcirculation assessment and jejunal tonometry

In-vivo microscopy of the sublingual mucosa was performed using the orthogonal polarization spectral (OPS) technique (MicroScan®, MicroVision Medical Inc.). Five sequences of 20 seconds each were recorded at each time point using a digital image conversion device. The sequences were analysed for vessel density and blood flow using AVA 3.0 software.

A tonometer tube with a silicone rubber balloon (catheter TRIP NGS, Tonometrics, Worcester) was inserted into the jejunum via a laparotomy to measure intestinal mucosal carbon dioxide ($PrCO_2$) using air-automated tonometry (Tonocap, Datex, Helsinki). Arterial pH and $PaCO_2$ values measured at the same time were used to calculate the intestinal pH (pHi) and intestinal mucosal-to-arterial carbon dioxide pressure difference ($Pr\text{-}aCO_2$).

Biological markers of inflammation

The blood samples were centrifuged at 2,000 rpm for 10 minutes at 4 °C. The plasma was stored at -80 °C until analysis. The plasma concentrations of tumour necrosis factor alpha (TNF-α) and interleukins 1β (IL-1β), 6 (IL-6), and 10 (IL-10) were measured using enzyme linked immunosorbent (ELISA) assays according to the manufacturer's instructions (DuoSet®, ELISA Development System, R&D Systems, Minneapolis, United States of America). The plasma levels of each cytokine were obtained through optical density measurements, and the absorbance was converted to pg/mL using a nonlinear regression curve and a standard curve.

Cortisol was measured using commercial immunoassay kits (Autodelfia Cortisol Kit, Wallac, Finland).

Data acquisition

The hemodynamic, jejunal tonometry, and blood gas data were measured prior to the bacterial infusion (baseline); immediately after infusion (T0); and 60 (T60), 120 (T120), 180 (T180), and 240 minutes (T240) later. Blood samples for cytokine measurements were obtained at baseline, T0, T60, and T240. The sublingual OPS images were recorded at baseline, T0, and T240 (Fig. 1). At the end of the experiment, isoflurane was increased to 5%, and the animals were euthanized via administration of an intravenous injection of potassium chloride.

Statistical analysis

The sample size for paired data was calculated using power analysis. A minimum of eight pigs per group was required to have a 95% chance (with 5% risk) to detect a difference of 3.5 mmol/L in blood lactate between groups, considering a standard deviation of 2 mmol/L. All data were assessed for normality using a D'Agostino-Pearson's test. Body weight, urine output, fluid volume, and norepinephrine consumption were compared between groups using one-way analysis of variance (ANOVA) and Student's t test. Normally distributed data were analysed within groups and among groups using two-way ANOVA for repeated measures (Sham vs. Shock and Dex-Shock, and then Shock vs. Dex-Shock) with a *post hoc* Tukey's test when appropriate. Non-normally distributed data were compared within groups using a Friedman's test with a *post hoc* Dunn's test, and the analysis between groups was performed using a Kruskal-Wallis and *post hoc* Dunn's test. Statistical significance was defined as $p < 0.05$. All tests were performed using a statistical software (Prism 6 for Windows, GraphPad). The results are presented as the mean ± standard deviation or median (interquartile range).

Results

Body weight was not significantly different between groups (Sham: 24.3 ± 2.6 kg; Shock: 24.7 ± 3 kg; Dex-Shock: 23.4 ± 1.3 kg, $p = 0.5558$).

Septic shock-induced disorders

Fluid loading, norepinephrine administration, and urinary output

The Sham group did not require additional fluid therapy or norepinephrine infusion throughout the study (Fig. 2). The fluid requirement (100 ± 22 mL/kg), and norepinephrine consumption (64.15 ± 93.4 µg/kg) were significantly higher in the Shock group than in the Sham group (50 ± 0 mL/kg and 0 µg/kg, respectively). There was no significant difference in urine output between groups ($p = 0.0757$).

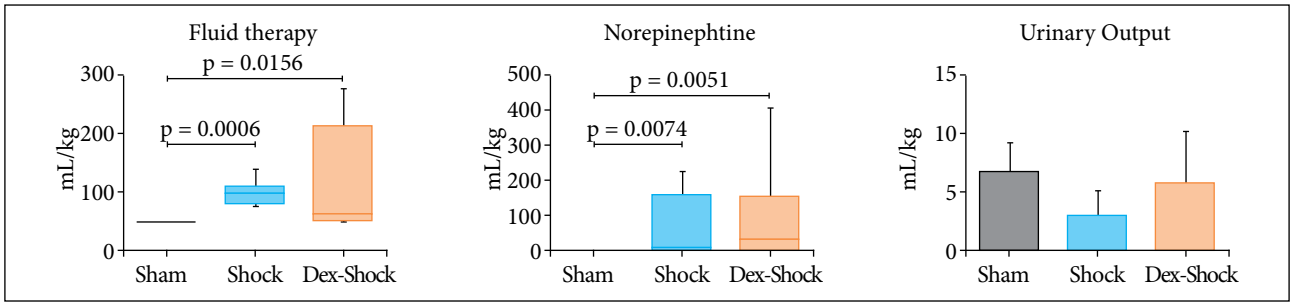
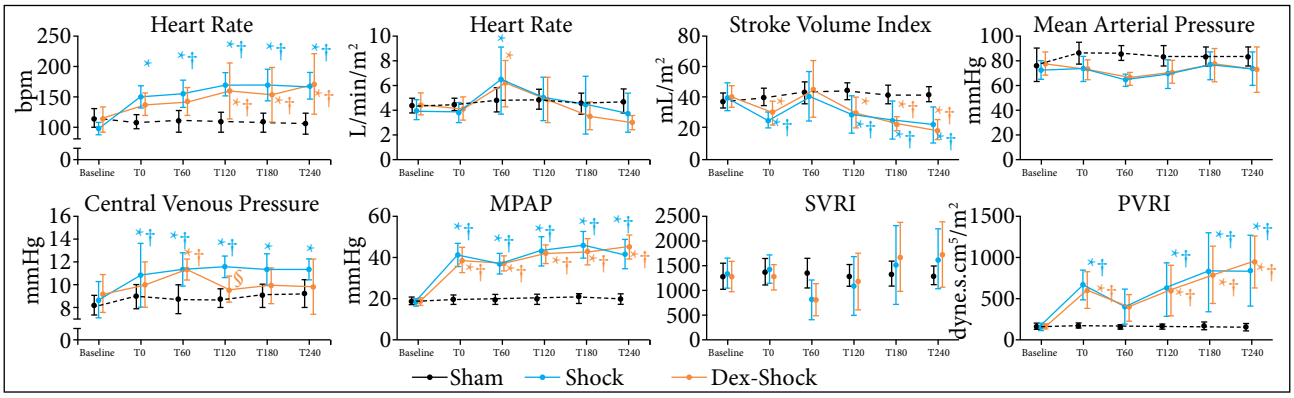


Figure 2 - Fluid loading, total dose of norepinephrine, and urinary output in anaesthetised pigs (Sham group), septic shock animals (Shock group), and septic shock animals receiving dexmedetomidine (Dex-Shock group).

Hemodynamic and microcirculation disorders

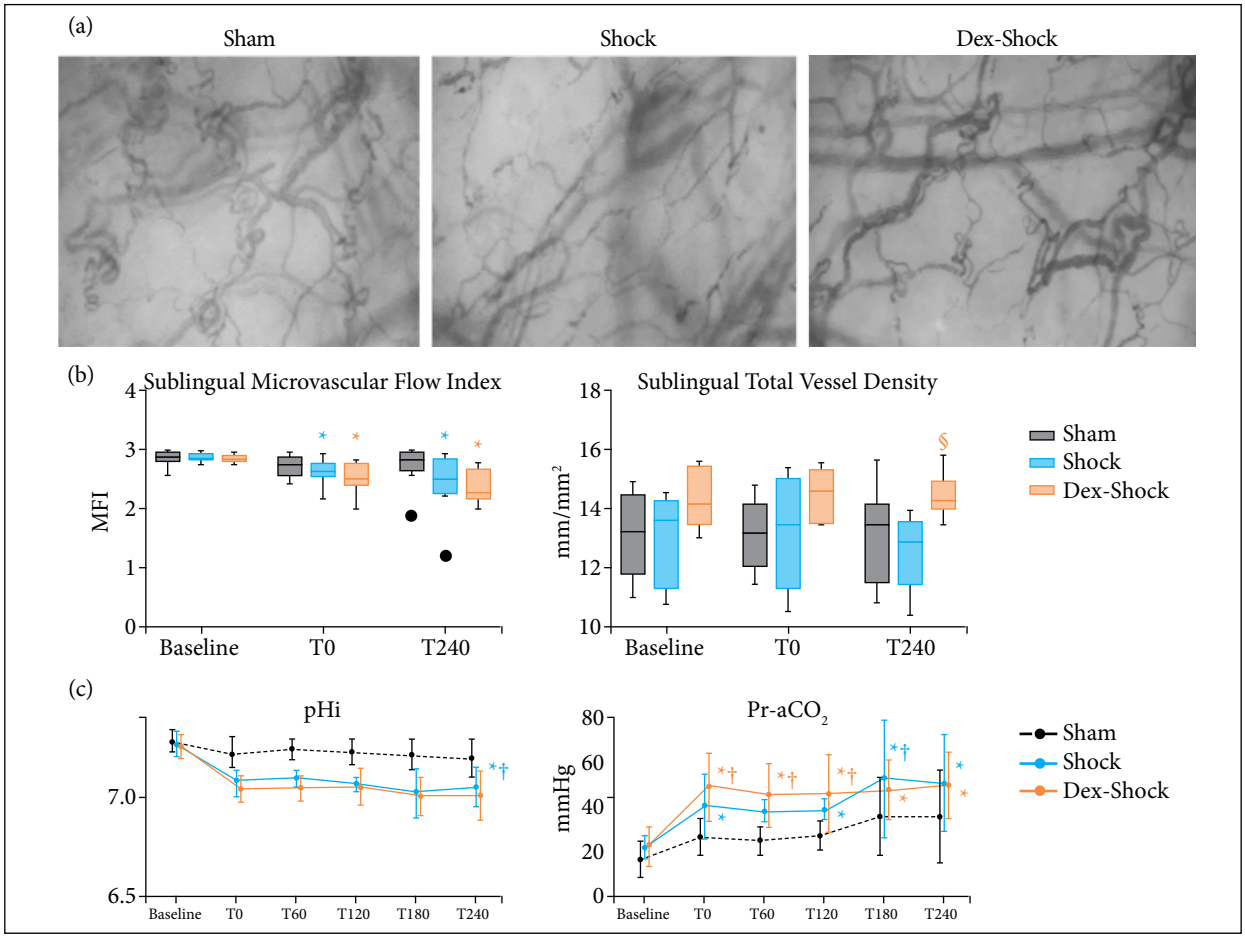
The Shock group had a significant increase (at T0, T60, T120, T180, and T240) in HR, MPAP, CVP, and PVRI, and a significant decrease in SVI (at T0, T120, T180, and T240) compared with baseline (Fig. 3). The SVRI was significantly decreased only at T60 in the Shock group ($p = 0.0424$). The CI increased significantly only at T60 in the Shock group ($p = 0.0002$). No significant hemodynamic changes were observed in Sham animals.

Sublingual blood flow was significantly reduced by septic shock ($p = 0.008$), whereas vessel density was not altered ($p = 0.2851$) (Fig. 4). The intestinal regional pH decreased significantly in the Shock group ($p < 0.0001$). Sham animals did not show any significant microcirculatory changes.



MPAP: mean pulmonary artery pressure; SVRI: systemic vascular resistance index; PVRI: pulmonary vascular resistance index; *$p < 0.05$ vs. baseline; † $p < 0.05$ vs. Sham.

Figure 3 - Hemodynamic changes in anesthetized pigs (Sham group), septic shock animals (Shock group), and septic shock animals administered dexmedetomidine (Dex-Shock group) before bacterial infusion (baseline); at the end of bacterial infusion (T0); and 60 (T60), 120 (T120), 180 (T180), and 240 minutes (T240) after bacterial infusion.



*p < 0.05 vs. baseline; † $p < 0.05$ vs. Sham group; § $p < 0.05$ vs. Shock group.

Figure 4 - Sublingual microcirculation and jejunal tonometry in anesthetized pigs (Sham group), septic shock animals (Shock group), and septic shock animals administered a dexmedetomidine infusion (Dex-Shock group). **(a)** An illustrative example for sublingual vessels density. **(b)** The median and 25-75 percentile values for sublingual blood flow and vessel density before bacterial infusion, at the end of bacterial infusion (T0), and 240 minutes (T240) after bacterial infusion. **(c)** The mean values for intestinal pH (pHi) and intestinal mucosal-to-arterial carbon dioxide pressure difference (Pr-aCO_2).

Systemic oxygenation, blood gas and electrolytes

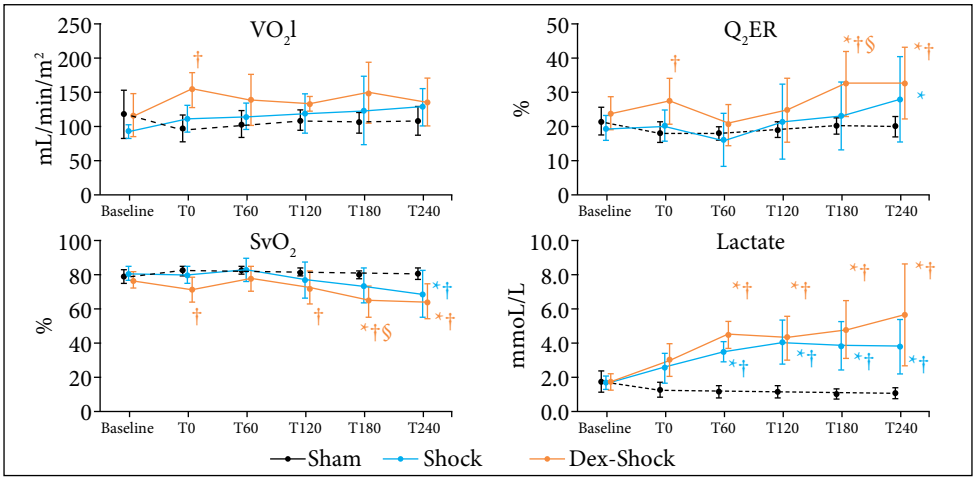
Septic shock resulted in a significant decrease in the arterial pH (from T0 to T240), the PaO_2/FiO_2 ratio (from T0 to T240), and plasma bicarbonate (from T60 to T240), and a significant increase in the plasma lactate (from T60 to T240), K^+ (T240), and haematocrit (at T0, T120, T180 and T240) compared with baseline (Table 1 and Fig. 5). These variables were also significantly different compared with those in Sham animals.

Blood glucose was not significantly changed in the Shock group compared with baseline. In the Sham group, blood glucose showed a slight increase at T60 compared with baseline. No significant differences in blood glucose were observed between Sham group and both Shock groups.

Table 1 - Blood gas and electrolytes.

	Groups	Baseline	T0	T60	T120	T180	T240	ANOVA P value
PaO_2/FiO_2 (mmHg)	Sham	556 ± 45	561 ± 70	549 ± 58	534 ± 56	532 ± 38	535 ± 50	Int <0.0001
	Shock	553 ± 42	408 ± 76*†	353 ± 123*†	309 ± 128*†	313 ± 163*†	315 ± 165*†	Time <0.0001
	Dex-Shock	570 ± 38	373 ± 123*†	316 ± 128*†	304 ± 176*†	306 ± 166*†	295 ± 166*†	Group 0.0015
$PaCO_2$ (mmHg)	Sham	41.7 ± 4.1	38.8 ± 2.8	40.6 ± 2.6	40.6 ± 3.5	39.7 ± 3.3	40.8 ± 3.0	Int 0.0329
	Shock	39.0 ± 5.6	45.7 ± 2.4	45.5 ± 2.4	43.8 ± 2.6	44.0 ± 5.0	45.8 ± 7.8	Time 0.0023
	Dex-Shock	40.9 ± 3.4	48.4 ± 5.9*†	46.7 ± 6.2	45.7 ± 7.2	45.3 ± 7.4	49.3 ± 8.2*	Group 0.0141
BIC (mmol/L)	Sham	26.0 ± 1.1	25.6 ± 3.2	27.2 ± 2.1	27.2 ± 2.7	26.2 ± 2.8	27.1 ± 2.1	Int <0.0001
	Shock	26.8 ± 2.7	24.7 ± 2.2	24.1 ± 2.4*†	22.7 ± 2.2*†	22.3 ± 3.0*†	22.8 ± 3.3*†	Time <0.0001
	Dex-Shock	27.2 ± 2.2	24.5 ± 2.0*	23.7 ± 1.7*†	23.4 ± 1.5*†	21.9 ± 2.4*†	21.7 ± 4.0*†	Group 0.0171
K (mmol/L)	Sham	3.8 ± 0.3	3.7 ± 0.4	3.9 ± 0.3	3.9 ± 0.4	3.9 ± 0.6	4.2 ± 0.5	Int <0.0001
	Shock	3.8 ± 0.4	3.8 ± 0.4	3.6 ± 0.4	4.0 ± 0.5	4.2 ± 0.5	4.9 ± 0.8*	Time <0.0001
	Dex-Shock	3.7 ± 0.4	4.0 ± 0.5	3.8 ± 0.5	4.1 ± 0.5	4.5 ± 0.5*	5.3 ± 0.7*†	Group 0.3065
Blood Glucose (mg/dL)	Sham	78.6 ± 19.9	85.8 ± 8.9	108.6 ± 29.1*	81.5 ± 2.1	89.0 ± 7.3	80.2 ± 7.5	Int 0.0058
	Shock	83.0 ± 22.9	88.4 ± 22.9	82.1 ± 34.4	74.2 ± 29.2	64.6 ± 29.6	69.8 ± 40.3	Time <0.0001
	Dex-Shock	71.0 ± 18.0	85.6 ± 25.3	63.6 ± 16.5†	52.1 ± 18.1	53.6 ± 17.7	45.2 ± 15.4	Group 0.0240
Haematocrit (%)	Sham	28.1 ± 2.9	26.8 ± 1.8	27.0 ± 2.8	26.8 ± 2.5	26.0 ± 1.8	25.8 ± 2.5	Int <0.0001
	Shock	27.5 ± 2.6	33.9 ± 3.2*†	29.4 ± 2.5	30.8 ± 2.6†	33.6 ± 4.6*†	36.6 ± 6.1*†	Time <0.0001
	Dex-Shock	24.8 ± 2.8	31.2 ± 2.3*†	26.8 ± 3.1	29.8 ± 1.8*	32.1 ± 3.8*†	34.2 ± 4.6*†	Group 0.0013

*P < 0.05 compared to baseline; † P < 0.05 compared to Sham group.



*p < 0.05 vs. baseline; † p < 0.05 vs. Sham; § p < 0.05 vs. Shock.

Figure 5 - Changes in oxygen consumption (VO_2I), the oxygen extraction ratio (O_2ER), mixed venous oxygen saturation (SvO_2), and plasma lactate in anaesthetised pigs (Sham group), septic shock animals (Shock group), and septic shock plus dexmedetomidine infusion animals (Dex-Shock group) at baseline; at the end of bacterial infusion (T0); and after 60 (T60), 120 (T120), 180 (T180), and 240 minutes (T240) after bacterial infusion.

Inflammatory markers

Animals in the Shock group exhibited a significant increase in the plasma levels of TNF-α (T0, T60, and T240), IL-1β (T240), IL-6 (T60 and T240), IL-10 (T0), and cortisol (T0 and T240) compared with baseline (Table 2). Sham animals showed no significant changes in inflammatory markers.

Table 2 - Plasma cytokines and cortisol.

	Groups	Baseline	T0	T60	T240	Friedman P-value
TNF-α (pg/mL)	Sham	91 (0; 115.5)	171.9 (140.4; 228.8)	144 (90.2; 210.6)	68.5 (13.6; 103.3)	0.0281
	Shock	44.1 (0.0; 105.7)	1,685 (1,673; 1,692)*†	1,684 (1,675; 1,685)*†	1,673 (1,640; 1,684)†	0.0003
	Dex-Shock	65.7 (30.8; 111.7)	1,685 (1,676; 1,689)*†	1,685 (1,681; 1,686)*†	1,658 (1,609; 1,667)†	< 0.0001
IL-1β (pg/mL)	Sham	0 (0.0; 24.1)	0 (0; 27.6)	0 (0; 0)	0 (0; 3.8)	0.9063
	Shock	0 (0; 0)	12.5 (0; 38.9)	86.8 (52.2; 267.9)†	663.9 (185; 1,245)*†	< 0.0001
	Dex-Shock	0 (0; 0)	1.9 (0; 13.3)	91.9 (61; 243.1)†	555.5 (114.2; 1,152)*†	0.0001
IL-6 (pg/mL)	Sham	0 (0; 5.7)	0 (0; 0)	0 (0; 0.5)	0 (0; 0)	> 0.999
	Shock	0 (0; 0)	144.9 (74.6; 319.6)†	1,922 (1,430; 2,463)*†	2,688 (2,005; 2,969)*†	< 0.0001
	Dex-Shock	0 (0; 4.1)	112.8 (51.8; 460.8)†	1,431 (1,172; 1,968)*†	1,590 (510.1; 2,321)*†	0.0006
IL-10 (pg/mL)	Sham	0 (0; 21)	0 (0; 0)	0 (0; 0.8)	0 (0; 6.8)	0.500
	Shock	0 (0; 7)	102.3 (83.8; 129)*†	37.8 (25.6; 75.3)†	56.2 (25; 120.6)†	0.0002
	Dex-Shock	0 (0; 0)	102.9 (30.2; 121.5)*†	29.6 (19.5; 56.3)†	35.3 (15.8; 56.9)	0.002
Cortisol (mcg/mL)	Sham	54.4 (37.2; 126.3)	91.8 (41.5; 140.1)	69.3 (55.2; 144.8)	49.5 (32.1; 109.7)	0.522
	Shock	138.7 (47.3; 150.4)	160.6 (154.2; 161.9)*†	159.4 (149.8; 164.2)†	154.8 (132.7; 166.7)*†	0.006
	Dex-Shock	67.7 (37.1; 135.8)	145.2 (134.2; 153.6)*	138 (129.1; 156.8)	146.9 (141.1; 158.8)*†	0.004

* P < 0.05 compared to baseline; † P < 0.05 compared to Sham group; TNF-α: tumour necrosis factor alpha; IL: interleukin.

Impact of dexmedetomidine on septic shock-induced disorders

Fluid loading, norepinephrine administration, and urinary output

Fluid loading ($p = 0.5848$), norepinephrine requirements ($p = 0.8438$), and urine output ($p = 0.1916$) were not significantly different between the Shock and Dex-Shock groups (Fig. 2).

Hemodynamic and microcirculatory disorders

The infusion of dexmedetomidine did not modify septic shock-induced cardiorespiratory disorders or sublingual blood flow (Table 1 and Fig. 3). However, the blood vessel density was significantly higher at T240 in the Dex-Shock group than in the untreated Shock group ($p = 0.0126$; Fig. 4).

Systemic oxygenation, blood gas, and electrolytes

Septic shock resulted in a decrease in the arterial pH, the PaO_2/FiO_2 ratio, plasma bicarbonate, and pHi, and an increase in O_2ER, the haematocrit, and K^+ (Table 1 and Fig. 5). These alterations were not significantly modified by the intravenous infusion of dexmedetomidine.

Inflammatory markers

Dexmedetomidine did not modify the septic shock-induced increase in TNF-α, IL-1β, IL-6, IL-10, or cortisol (Table 2).

Discussion

In this study, performed in anesthetized pigs injected with live *E. coli* and monitored over 4 hours, dexmedetomidine did not impact norepinephrine requirements; did not mitigate or worsen septic shock-induced hemodynamic disorders; promoted a slight increase in impact sublingual vessel density, and induced an initial and transitory increase in oxygen consumption and a late decrease in mixed venous O_2 saturation associated with a slight but significant increase in lactate.

The intravenous administration of live *E. coli* caused septic shock characterized by an immediate decrease in the MAP requiring norepinephrine administration, a severe reduction in urinary output, and an initial hyperdynamic state followed by a progressive and continuous decrease in the cardiac index. Dexmedetomidine did not modify norepinephrine requirement, but preserved urinary output. This result confirms previous experimental and clinical studies reporting that dexmedetomidine can offer a protective effect against septic[14] and postoperative acute kidney injury[15,16] by exerting an anti-inflammatory effect and ischemia/reperfusion attenuation. Dexmedetomidine also inhibits the release of vasopressin and insulin, increasing urinary output and blood glucose[17,18]. Because α2-agonists improve the pressor response to norepinephrine[19], reduced vasoactive drug requirements during septic shock have been reported in patients sedated by dexmedetomidine[20]. However, this benefit is inconsistently observed[21] and was not documented in the present study. Dexmedetomidine reversed the septic shock-induced increase in CVP from the second hour following bacterial injection, and did not modify the other septic shock-induced hemodynamic disorders. The alterations in sublingual and intestinal microcirculation induced by septic shock[1] were not worsened by dexmedetomidine. Additionally, in the present study, dexmedetomidine did not prevent the decrease in sublingual blood flow, but significantly increased vessel density. This finding is in accordance with a previous study showing that dexmedetomidine attenuates the microcirculatory derangements associated with experimental sepsis[22]. The mechanisms are not fully understood, but leukocyte rolling, and adhesion may be involved[22].

Despite the lack of improvement in sublingual and intestinal blood flows, dexmedetomidine induced an initial tissue O_2 impairment reflected by a significant increase in O_2ER and a late increase in lactate associated with a significant decrease in SvO_2. This result is more relevant because dexmedetomidine increases lactate clearance in patients with septic shock[23]. SvO_2 has been shown to be a surrogate for the cardiac index, a target for hemodynamic therapy[24]. Accordingly, in the present study, the decrease in SvO_2 in the Dex-Shock group reflected the decrease in the cardiac index, which was higher in the middle of the experiment, but decreased at the end of it. Although the current dose of dexmedetomidine does not modify the cardiac index in healthy animals[25], it might have an impact in the presence of sepsis[19]. In addition, the haemoconcentration caused by fluid extravasation from the microcirculation may have contributed to the development of a compensatory increase in oxygen extraction, which consequently led to the SvO_2 and cardiac index decrease. The deterioration of systemic oxygenation was accompanied by changes in arterial lactate and pH, which were consistent with metabolic lactic acidosis. As attested by the development of splanchnic acidosis, tissue oxygenation was impaired in all septic animals, confirming a previous study[10]. However, dexmedetomidine did not further affect the intestinal pH, $PrCO_2$, or $Pr\text{-}aCO_2$, as previously reported in septic patients[26]. Therefore, our data do not allow us to identify dexmedetomidine-induced tissue O_2 impairment.

As previously reported, the intravenous injection of live *E. coli* induced an increase in the pulmonary artery pressure and pulmonary vascular resistance[10,26]; a significant decrease in PaO_2/FiO_2, and a significant increase in $PaCO_2$, in contrast to several experimental studies reporting that dexmedetomidine attenuates endotoxin and ventilator-induced lung injury[27-29]. In our study, dexmedetomidine did not modify any of the respiratory disorders resulting from the intravenous injection of live *E. coli*.

Intravenous injection of *E. coli* also induces the release of inflammatory cytokines[30,31]. In our study, dexmedetomidine did not modify cytokine release, although previous experimental studies reported a dose-dependent decrease in TNF-α and IL-6 in an endotoxin-induced shock model[5,32]. *In vitro*, dexmedetomidine failed to influence the cytokine levels and neutrophil function associated with chemotaxis, phagocytosis, or superoxide production after *E. coli* exposure[33].

However, in a clinical trial, septic patients sedated with dexmedetomidine had lower levels of TNF-α, IL-1β, and IL-6 detected 24 hours after admission to the ICU[26]. Therefore, the lack of a significant reduction in cytokine levels in the present study may be related to the insufficient assessment time after *E. coli* infusion. In addition, the cytokine response

is characterized by large individual variability, and the absence of a significant impact of dexmedetomidine on IL-6 and IL-10 might be related to insufficient power, as previously reported[34].

Conclusions

Dexmedetomidine did not affect the early hemodynamic, metabolic, and inflammatory disorders induced by septic shock. However, a late mismatch between oxygen supply and demand was observed in animals receiving dexmedetomidine, which can also be caused by cardiac output reduction. Finally, dexmedetomidine preserved the sublingual microcirculatory vessel density, but it did not protect against septic shock-induced decrease in sublingual blood flow. Therefore, the results of the present study suggest that dexmedetomidine should be used cautiously in septic shock patients.

Authors' contribution

Conception the study: Monteiro Filho A; **Design of the study:** Auler Jr. JOC and Fantoni DT; **Conception and design of the study:** Carnicelli P and Otsuki DA; **Analysis of data:** Carnicelli P, Otsuki DA, Ida KK, Auler Jr. JOC, Rouby JJ and Fantoni DT; **Technical procedures:** Kahvegian MAP; **Manuscript writing:** Otsuki DA and Ida KK; **Critical revision:** Rouby JJ; **Final approval the version to be published:** Auler Jr. JOC and Fantoni DT.

Data availability statement

Data will be available upon request.

Funding

Fundação de Amparo . Pesquisa do Estado de São Paulo.

[https://doi.org/10.13039/501100001807]

Grant nº 08/58875-4

Acknowledgments

Not applicable.

References

1. De Backer D, Ricottilli F, Ospina-Tascón GA. Septic shock: a microcirculation disease. Curr Opin Anesthesiol. 2021;34(2):85-91. https://doi.org/10.1097/ACO.0000000000000957
2. Sakr Y, Dubois MJ, De Backer D, Creteur J, Vincent JL. Persistent microcirculatory alterations are associated with organ failure and death in patients with septic shock. Crit Care Med. 2004;32(9):1825-31. https://doi.org/10.1097/01.ccm.0000138558.16257.3f
3. Szumita PM, Baroletti SA, Anger KE, Wechsler ME. Sedation and analgesia in the intensive care unit: evaluating the role of dexmedetomidine. Am J Health Syst Pharm. 2007;64(1):37-44. https://doi.org/10.2146/ajhp050508
4. Blaudszun G, Lysakowski C, Elia N, Tramèr MR. Effect of perioperative systemic alpha2 agonists on postoperative morphine consumption and pain intensity: systematic review and meta-analysis of randomized controlled trials. Anesthesiology. 2012;116:1312-22. https://doi.org/10.1097/aln.0b013e31825681cb

5. Taniguchi T, Kidani Y, Kanakura H, Takemoto Y, Yamamoto K. Effects of dexmedetomidine on mortality rate and inflammatory responses to endotoxin-induced shock in rats. Crit Care Med. 2004;32(6):1322-6. https://doi.org/10.1097/01.ccm.0000128579.84228.2a

6. Lee JM, Han HJ, Choi WK, Yoo S, Baek S, Lee J. Immunomodulatory effects of intraoperative dexmedetomidine on T helper 1, T helper 2, T helper 17 and regulatory T cells cytokine levels and their balance: a prospective, randomised, double-blind, dose-response clinical study. BMC Anesthesiol. 2018;18:164. https://doi.org/10.1186/s12871-018-0625-2

7. Ebert TJ, Hall JE, Barney JA, Uhrich TD, Colinco MD. The effects of increasing plasma concentrations of dexmedetomidine in humans. Anesthesiology. 2000;93:382-94. https://doi.org/10.1097/00000542-200008000-00016

8. Su X, Meng ZT, Wu XH, Cui F, Li HL, Wang DX, Zhu X, Zhu SN, Maze M, Ma D. Dexmedetomidine for prevention of delirium in elderly patients after non-cardiac surgery: a randomised, double-blind, placebo-controlled trial. Lancet. 2016;388(10054):1893-902. https://doi.org/10.1016/S0140-6736(16)30580-3

9. Skrobik Y, Duprey MS, Hill NS, Devlin JW. Low-dose nocturnal dexmedetomidine prevents ICU delirium. A randomized, placebo-controlled trial. Am J Respir Crit Care Med. 2018;197(9):1147-56. https://doi.org/10.1164/rccm.201710-1995OC

10. Garrido AG, Poli de Figueiredo LF, Cruz RJ Jr., Silva E, Rocha E Silva M. Short-lasting systemic and regional benefits of early crystalloid infusion after intravenous inoculation of dogs with live Escherichia coli. Braz J Med Biol Res. 2005;38(6):873-84. https://doi.org/10.1590/s0100-879x2005000600009

11. Rahal L, Garrido AG, Cruz RJ Jr, Silva E, Poli-de-Figueiredo LF. Fluid replacement with hypertonic or isotonic solutions guided by mixed venous oxygen saturation in experimental hypodynamic sepsis. J Trauma. 2009;67(6):1205-12. https://doi.org/10.1097/TA.0b013e31818b2567

12. Dellinger RP, Levy MM, Carlet JM, Bion J, Parker MM, Jaeschke R, Reinhart K, Angus DC, Brun-Buisson C, Beale R, Calandra T, Dhainaut JF, Gerlach H, Harvey M, Marini JJ, Marshall J, Ranieri M, Ramsay G, Sevransky J, Thompson BT, Townsend S, Vender JS, Zimmerman JL, Vincent JL. Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock. Crit Care Med. 2008;36(1):296-327. https://doi.org/10.1097/01.CCM.0000298158.12101.41

13. Holt JP, Rhode EA, Kines H. Ventricular volumes and body weight in mammals. Am J Physiol. 1968;215:704-15. HTTPS://DOI.ORG/10.1152/ajplegacy.1968.215.3.704.

14. Qiu R, Yao W, Ji H, Yuan D, Gao X, Sha W, Wang F, Huang P, Hei Z. Dexmedetomidine restores septic renal function via promoting inflammation resolution in a rat sepsis model. Life Sci. 2018;204:1-8. https://doi.org/10.1016/j.lfs.2018.05.001

15. Cho JS, Shim JK, Soh S, Kim MK, Kwak YL. Perioperative dexmedetomidine reduces the incidence and severity of acute kidney injury following valvular heart surgery. Kidney Int. 2016;89(3):693-700. https://doi.org/10.1038/ki.2015.306

16. Liu Y, Sheng B, Wang S, Lu F, Zhen J, Chen W. Dexmedetomidine prevents acute kidney injury after adult cardiac surgery: a meta-analysis of randomized controlled trials. BMC Anesthesiol. 2018;18:7. https://doi.org/10.1186/s12871-018-0472-1

17. Sinclair MD. A review of the physiological effects of alpha2-agonists related to the clinical use of medetomidine in small animal practice. Can Vet J. 2003;44(11):885-97.

18. Villela NR, Nascimento Júnior P, Carvalho LR, Teixeira A. Effects of dexmedetomidine on renal system and on vasopressin plasma levels. Experimental study in dogs. Rev Bras Anestesiol. 2005;55(4):429-40. https://doi.org/10.1590/s0034-70942005000400007

19. Geloen A, Chapelier K, Cividjian A, Dantony E, Rabilloud M, May CN, Quintin L. Clonidine and dexmedetomidine increase the pressor response to norepinephrine in experimental sepsis: a pilot study. Crit Care Med. 2013;41(12):e431-8. https://doi.org/10.1097/CCM.0b013e3182986248

20. Morelli A, Sanfilippo F, Arnemann P, Hessler M, Kampmeier TG, D'Egidio A, Orecchioni A, Santonocito C, Frati G, Greco E, Westphal M, Rehberg SW, Ertmer C. The effect of propofol and dexmedetomidine sedation on norepinephrine requirements in septic shock patients: a crossover trial. Crit Care Med. 2019;47(2):e89-e95. https://doi.org/10.1097/CCM.0000000000003520

21. Nelson KM, Patel GP, Hammond DA. Effects from continuous infusions of dexmedetomidine and propofol on hemodynamic stability in critically Ill adult patients with septic shock. J Intensive Care Med. 2020;35(9):875-80. https://doi.org/10.1177/0885066618802269

22. Miranda ML, Balarini MM, Bouskela E. Dexmedetomidine attenuates the microcirculatory derangements evoked by experimental sepsis. Anesthesiology. 2015;122:619-30. https://doi.org/10.1097/ALN.0000000000000491

23. Miyamoto K, Nakashima T, Shima N, Kato S, Ueda K, Kawazoe Y, Ohta Y, Morimoto T, Yamamura H; DESIRE Trial Investigators. Effect of dexmedetomidine on lactate clearance in patients with septic shock: a subanalysis of a multicenter randomized controlled trial. Shock. 2018;50(2):162-6. https://doi.org/10.1097/SHK.0000000000001055

24. Gattinoni L, Brazzi L, Pelosi P, Latini R, Tognoni G, Pesenti A, Fumagalli R. A trial of goal-oriented hemodynamic therapy in critically ill patients. SvO2 Collaborative Group. N Engl J Med. 1995;333:1025-32. https://doi.org/10.1056/NEJM199510193331601

25. Pascoe PJ. The cardiopulmonary effects of dexmedetomidine infusions in dogs during isoflurane anesthesia. Vet Anaesth Analg. 2015;42(4):360-8. https://doi.org/10.1111/vaa.12220

26. Memiş D, Hekimoğlu S, Vatan I, Yandim T, Yüksel M, Süt N. Effects of midazolam and dexmedetomidine on inflammatory responses and gastric intramucosal pH to sepsis, in critically ill patients. Br J Anaesth. 2007;98(4):550-2. https://doi.org/10.1093/bja/aem017

27. Chen H, Sun X, Yang X, Hou Y, Yu X, Wang Y, Wu J, Liu D, Wang H, Yu J, Yi W. Dexmedetomidine reduces ventilator-induced lung injury (VILI) by inhibiting Toll-like receptor 4 (TLR4)/nuclear factor (NF)-κB signaling pathway. Bosn J Basic Med Sci. 2018;18(2):162-9. https://doi.org/10.17305/bjbms.2018.2400

28. Yang CL, Chen CH, Tsai PS, Wang TY, Huang CJ. Protective effects of dexmedetomidine-ketamine combination against ventilator-induced lung injury in endotoxemia rats. J Surg Res. 2011;167(2):e273-81. https://doi.org/10.1016/j.jss.2010.02.020

29. Yang CL, Tsai PS, Huang CJ. Effects of dexmedetomidine on regulating pulmonary inflammation in a rat model of ventilator-induced lung injury. Acta Anaesthesiol Taiwan. 2008;46(4):151-9. https://doi.org/10.1016/S1875-4597(09)60002-3

30. Calzavacca P, Booth LC, Lankadeva YR, Bailey SR, Burrell LM, Bailey M, Bellomo R, May CN. Effects of clonidine on the cardiovascular, renal, and Inflammatory responses to experimental bacteremia. Shock. 2019;51(3):348-55. https://doi.org/10.1097/SHK.0000000000001134

31. Thorgersen EB, Hellerud BC, Nielsen EW, Barratt-Due A, Fure H, Lindstad JK, Pharo A, Fosse E, Tønnessen TI, Johansen HT, Castellheim A, Mollnes TE. CD14 inhibition efficiently attenuates early inflammatory and hemostatic responses in Escherichia coli sepsis in pigs. FASEB J. 2010;24(3):712-22. https://doi.org/10.1096/fj.09-140798

32. Taniguchi T, Kurita A, Kobayashi K, Yamamoto K, Inaba H. Dose- and time-related effects of dexmedetomidine on mortality and inflammatory responses to endotoxin-induced shock in rats. J Anesth. 2008;22:221-8. https://doi.org/10.1007/s00540-008-0611-9

33. Nishina K, Akamatsu H, Mikawa K, Shiga M, Maekawa N, Obara H, Niwa Y. The effects of clonidine and dexmedetomidine on human neutrophil functions. Anesth Analg. 1999;88(2):452-8. https://doi.org/10.1097/00000539-199902000-00042

34. Venn RM, Bryant A, Hall GM, Grounds RM. Effects of dexmedetomidine on adrenocortical function, and the cardiovascular, endocrine and inflammatory responses in post-operative patients needing sedation in the intensive care unit. Br J Anaesth. 2001;86(5):650-6. https://doi.org/10.1093/bja/86.5.650