

Dexmedetomidine Attenuates the Microcirculatory Derangements Evoked by Experimental Sepsis

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ABSTRACT

Background: Dexmedetomidine, an α -2 adrenergic receptor agonist, has already been used in septic patients although few studies have examined its effects on microcirculatory dysfunction, which may play an important role in perpetuating sepsis syndrome. Therefore, the authors have designed a controlled experimental study to characterize the microcirculatory effects of dexmedetomidine in an endotoxemia rodent model that allows *in vivo* studies of microcirculation.

Methods: After skinfold chamber implantation, 49 golden Syrian hamsters were randomly allocated in five groups: (1) control animals; (2) nonendotoxemic animals treated with saline; (3) nonendotoxemic animals treated with dexmedetomidine ($5.0 \mu\text{g kg}^{-1} \text{ h}^{-1}$); (4) endotoxemic (lipopolysaccharide 1.0 mg/kg) animals treated with saline; and (5) endotoxemic animals treated with dexmedetomidine. Intravital microscopy of skinfold chamber preparations allowed quantitative analysis of microvascular variables and venular leukocyte rolling and adhesion. Mean arterial blood pressure, heart rate, arterial blood gases, and lactate concentrations were also documented.

Results: Lipopolysaccharide administration increased leukocyte rolling and adhesion and decreased capillary perfusion. Dexmedetomidine significantly attenuated these responses: compared with endotoxemic animals treated with saline, those treated with dexmedetomidine had less leukocyte rolling ($11.8 \pm 7.2\%$ *vs.* $24.3 \pm 15.0\%$; $P < 0.05$) and adhesion (237 ± 185 *vs.* 510 ± 363 ; $P < 0.05$) and greater functional capillary density ($57.4 \pm 11.2\%$ of baseline values *vs.* $45.9 \pm 11.2\%$; $P < 0.05$) and erythrocyte velocity ($68.7 \pm 17.6\%$ of baseline values *vs.* $54.4 \pm 14.8\%$; $P < 0.05$) at the end of the experiment.

Conclusions: Dexmedetomidine decreased lipopolysaccharide-induced leukocyte–endothelial interactions in the hamster skinfold chamber microcirculation. This was accompanied by a significant attenuation of capillary perfusion deficits, suggesting that dexmedetomidine yields beneficial effects on endotoxemic animals' microcirculation. (**ANESTHESIOLOGY 2015; 122:619-30**)

SEPSIS is a pathology highlighted by its high incidence, morbidity, mortality, and cost to healthcare system.^{1,2} Depending on its severity and duration, increased venular leukocyte adhesion and aggregation, microthrombosis, and microvascular vasoconstriction may occur, leading to ischemia, impaired organ perfusion and function, and death. Thus, drugs that assist in reversal of microcirculatory changes could be decisive in sepsis treatment.³

Many unique characteristics favor dexmedetomidine use during sepsis syndrome. This potent and highly selective α -2 adrenergic receptor agonist evokes sedation combined with arousability, preserves spontaneous ventilation, and has been associated with reduced incidence of delirium and increased pressor response to norepinephrine.⁴⁻⁷ Unfortunately, few studies have examined the influence of α -2 adrenergic receptor agonists on microcirculation and tissue perfusion in septic patients.

Dexmedetomidine has vasodilator effects that could assist in the recruitment of microcirculation.^{3,8} Furthermore, α -2 adrenergic receptor agonists have effects on immunity, inflammation, and coagulation.^{9,10} Together, these effects may contribute to restoration of microcirculatory function,

What We Already Know about This Topic

- Sepsis may produce ischemia and impaired organ function by increasing venular leukocyte adhesion and aggregation, microthrombosis, and microvascular vasoconstriction
- Dexmedetomidine can produce vasodilation and α -2 adrenergic agonists can affect immunity, inflammation, and coagulation

What This Article Tells Us That Is New

- Intravital microscopy of skinfold chamber microcirculation preparations in unanesthetized golden Syrian hamsters found that dexmedetomidine partially restored lipopolysaccharide-induced capillary perfusion deficits
- Dexmedetomidine attenuated both increases in venular leukocyte rolling and adhesion and decreases in functional capillary density and erythrocyte velocity induced by lipopolysaccharide

improving tissue perfusion, and reducing organ failure.^{11,12} Based on these findings, we hypothesized that dexmedetomidine could reverse the microcirculatory derangements evoked by sepsis. Thus, the current controlled experimental study was carried out to test this hypothesis in an endotoxemia rodent model that allows *in vivo* studies of the microcirculation.

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Materials and Methods

Experiments were performed on 49 male golden Syrian hamsters (*Mesocricetus auratus*; 95 to 105 g) housed one per cage under controlled conditions of light (12:12 h light–dark cycle) and temperature ($21.0^{\circ} \pm 1.0^{\circ}\text{C}$), with free access to water and standard chow. All procedures were approved by the Rio de Janeiro State University Animal Care and Use Committee, Rio de Janeiro, RJ, Brazil (protocol number CEUA/060/2010) and are consistent with the United States National Institutes of Health Guide for the Care and Use of Laboratory Animals.¹³

Animal Preparation

The chamber implantation procedure has been described previously by Endrich *et al.*¹⁴ in details and is briefly shown in figure 1. Six days after chamber implantation (recovery period), animals were reanesthetized and the left carotid artery was catheterized (polyethylene-50 catheter) allowing continuous mean arterial blood pressure (MAP) and heart rate (HR) monitoring and blood sampling. The left jugular vein was also catheterized (polyethylene-10 catheter) for fluid infusion and drug injection. These catheters were tunneled under the skin, exteriorized at the dorsal side of the neck, filled with heparinized saline solution (40 IU/ml), and attached to the chamber frame with tape. Experiments were performed on awake animals after 24 h of catheter implantation.

MAP, HR, and Temperature Monitoring

Mean arterial blood pressure was continuously monitored during the study period through the arterial catheter and a blood pressure transducer (TSD104A; BIOPAC Systems, Goleta, CA). Analog pressure signals were digitized (MP100 Data Acquisition System; BIOPAC Systems) and processed using data acquisition software for cardiovascular experiments (AcqKnowledge Software v. 3.5.7, BIOPAC Systems). The software automatically determined HR from the pressure trace and expressed it as beats per min. Rectal and skin-fold chamber temperatures were monitored with a beaded type K thermocouple probe (Wavetek 23XT, Wavetek Corporation, San Diego, CA). The body temperature of the hamsters was maintained with a heating pad placed near the animal controlled by a rectal thermistor (LB750, Uppsala Processdata AB, Uppsala, Sweden).

Intravital Microscopy

Intravital microscopy was performed as stated in figure 1. Acquired microcirculatory images were recorded as video files in digital media for later evaluation. Quantitative off-line analysis of all videos generated during the experiments was performed using Cap-Image 7.2, a computer-assisted image analysis system (Dr. Zeintl Biomedical Engineering, Heidelberg, Germany)¹⁵ by an investigator blinded to drug treatment. In each animal, 2 arterioles, 2 venules, and 10 capillary fields were chosen taking into account the absence of inflammation or bleeding in the microscopic field and the

presence of histological landmarks that could facilitate the subsequent return to the same field, since the same vessels and capillary fields were studied throughout the experiment. Arteriolar and venular mean internal diameters were measured as the perpendicular distance (in micrometers) between the vessel walls. Arteriolar and venular blood flow velocities were determined by a semiquantitative score using an ordinal scale¹⁶: 0, no flow; 1, intermittent flow; 2, sluggish flow; 3, normal flow; and 4, supranormal flow. The functional capillary density (FCD) was considered to be the total length (in centimeters) of spontaneously erythrocyte-perfused capillaries per square centimeter of tissue surface area (cm/cm^2). Erythrocyte velocity in capillaries (Erythrocyte-Vel) was assessed by frame-to-frame analysis and determined as the ratio between the distance traveled by an erythrocyte and the time required for this displacement (expressed as mm/s). One capillary per quadrant of capillary field was studied in each animal during Erythrocyte-Vel assessment (40 capillaries in total). The selection of these capillaries was based on two criteria: they should be representative of the mean Erythrocyte-Vel of their quadrant and have good image quality for reliable analysis.

Evaluation of Venular Leukocyte–Endothelial Interactions

Intravital fluorescence microscopy was performed as stated in figure 1. According to their interaction with the microvascular endothelium, leukocytes were classified as passing, rolling, and adherent. Passing leukocytes were defined as leukocytes traversing an observed venular segment without sticking contact (adherence) to the endothelium lining. The number of passing leukocytes was expressed as cells per minute. Rolling leukocytes were defined as leukocytes moving along the endothelial lining at a velocity slower than that of the surrounding erythrocytes. The number of rolling leukocytes was expressed as a percentage of the number of passing leukocytes. A leukocyte was considered to be adherent to the venular endothelium lining if it remained stationary for more than 30 s. Adherent cells were counted in a 100 μm venular segment and the number of adherent leukocytes was expressed as the number of adherent cells per square millimeter of endothelial surface (cells/mm^2), calculated from the venular mean internal diameter and length (100 μm), assuming cylindrical vessel geometry. One venule was studied in each animal, and a single period of 60 s was analyzed off-line for all cell counts.

Arterial Blood Gases and Lactate Analysis

Blood samples were withdrawn from the arterial catheter and immediately analyzed in a blood gas analyzer (CG4+ cartridge, i-STAT System, Abbott Laboratories, Abbott Park, IL) for pH, partial pressure of oxygen (PO_2), partial pressure of carbon dioxide (PCO_2), bicarbonate level (HCO_3^-), base excess (BE), total carbon dioxide content (TCO_2), arterial oxyhemoglobin saturation (SatO_2), and arterial lactate concentrations.

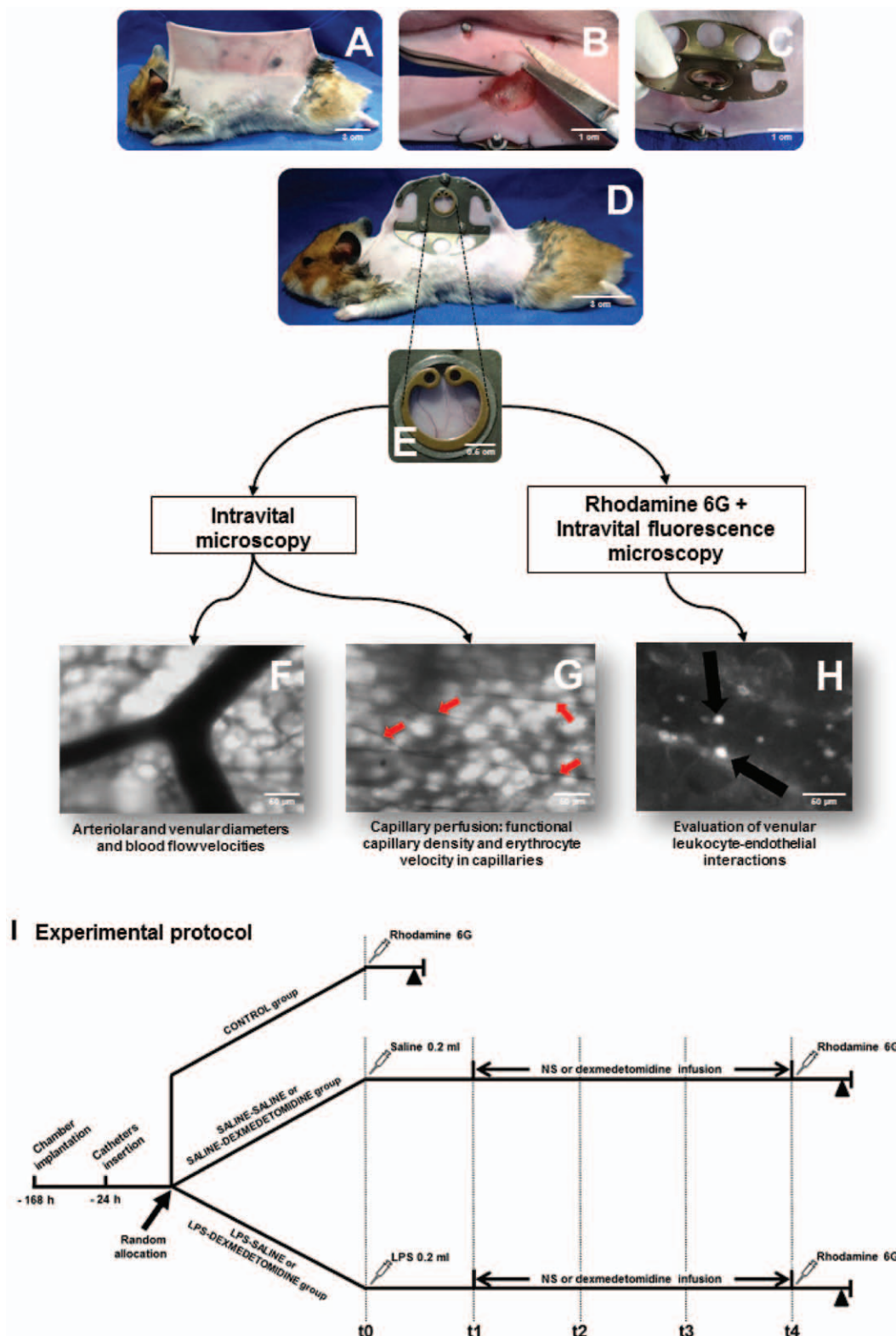


Fig. 1. Animal preparation, intravital microscopy setup, and experimental protocol. (A) During anesthesia with sodium pentobarbital (90 mg/kg intraperitoneal) animals' dorsal hair was depilated and the skin lifted away, creating a skinfold. (B) One layer of the skinfold was microscurgically excised in a circular area of 15 mm in diameter. (C and D) The remaining layer, consisting of epidermis, subcutaneous tissue, and thin striated skin muscle, was sandwiched between two titanium frames, one of which was covered with a circular cover glass, creating the window chamber (close-up view in E). After 7 days, unanesthetized animals were placed in a restraining plexiglass tube attached to an intravital microscope (Ortholux, Leitz, Wetzlar, Germany) equipped

Experimental Protocol

Animals were suitable for experiments if their baseline MAP, HR, and rectal temperature were within the normal range and if they showed no signs of inflammation or bleeding in the skinfold chamber. Included animals were randomly allocated in five groups: CONTROL (control animals; $n = 5$), SALINE–SALINE (nonendotoxemic animals treated with normal saline [NaCl 0.9%; NS]; $n = 10$), SALINE–DEXMEDETOMIDINE (nonendotoxemic animals treated with dexmedetomidine; $n = 10$), lipopolysaccharide–SALINE (endotoxemic animals treated with NS; $n = 12$), and lipopolysaccharide–DEXMEDETOMIDINE (endotoxemic animals treated with dexmedetomidine; $n = 12$).

After baseline determination of MAP, HR, and microcirculatory parameters, animals belonging to endotoxemic groups (lipopolysaccharide–SALINE and lipopolysaccharide–DEXMEDETOMIDINE) received an intravenous injection of 1.0 mg/kg (0.2 ml) of *Escherichia coli* serotype 055:B5 lipopolysaccharide (Sigma-Aldrich, St. Louis, MO). Nonendotoxemic groups (SALINE–SALINE and SALINE–DEXMEDETOMIDINE) received an intravenous injection of 0.2 ml of NS. One hour after lipopolysaccharide or NS administration, a continuous intravenous infusion of NS or dexmedetomidine solution ($5.0 \mu\text{g kg}^{-1} \text{h}^{-1}$) was initiated and maintained at a 0.1 ml/h infusion rate for 3 h.

As shown in figure 1, sequential measurements of MAP, HR, and microcirculatory parameters were performed at five

time points: at baseline (t_0), just before the initiation of the continuous intravenous infusion of NS or dexmedetomidine (t_1), and after 1 h (t_2), 2 h (t_3), and 3 h (t_4) of infusion. After 3 h of continuous NS or dexmedetomidine infusion, all animals received an intravenous injection of 0.15 mg/kg (0.4 ml) of rhodamine 6G. After a 10-min waiting period, animals were assessed by intravital fluorescence microscopy for evaluation of venular leukocyte–endothelial interactions. Blood sampling for arterial blood gases and lactate analysis was performed at the end of the study period.

Hamsters allocated in the CONTROL group served as baseline controls for leukocyte–endothelial interactions, lactate, and arterial blood gases parameters. To measure these parameters, animals were submitted to a partial experimental protocol: after baseline determination of MAP and HR, animals bypassed NS or lipopolysaccharide administration and NS or dexmedetomidine 3-h infusion protocol, going directly to rhodamine 6G administration (evaluation of leukocyte–endothelial interactions) and blood sampling.

After arterial blood sampling, all animals were sacrificed by an intravenous overdose of sodium pentobarbital ($>200 \text{ mg/kg}$; Hypnol 3%; Syntec, Cotia, SP, Brazil).

Statistical Analysis

Results are expressed as means \pm SD for each group, unless otherwise noted. Sample size was based on previous experience with the endotoxemia and microcirculation models used. Normally, with these models it is possible to observe significant differences between groups with the inclusion of 5 to 6 animals per group. In this study, a larger number of animals (10 to 12 in each group) was used because of the lack of data regarding the use of dexmedetomidine in association with the hamster skinfold window chamber model. Endotoxemic groups included a larger number of animals (12 *vs.* 10) considering the possibility of animal loss due to death during the experimental protocol. Statistical comparisons of normally distributed variables (assessed by Shapiro–Wilk test) were performed using two-way ANOVA for repeated measures and one way ANOVA as appropriate, whereas Friedman and Kruskal–Wallis tests were used for other variables. When appropriate, an adequate test was used for *post hoc* analysis: Bonferroni method or Dunn multiple comparisons. Venular leukocyte–endothelial interactions were correlated with capillary perfusion changes during the experiment, as depicted by percentage reductions of functional capillary density and erythrocyte velocity in capillaries values. These correlations were performed using Spearman rank correlation. All statistical analyses were performed using GraphPad Prism 6.03 (GraphPad Software, La Jolla, CA) and the significance level was set as P value less than 0.05 for a two-tailed test.

Results

The average body weight of hamsters was $100.7 \pm 3.2 \text{ g}$ with no significant differences among groups. All animals

Fig. 1. (continued) with an epifluorescence assembly (100-W HBO mercury lamp with filter blocks, Leitz). Moving images of the microcirculation were obtained using a 20 \times objective (CF SLWD Plan EPI 20 \times /0.35 Achromat Objective WD 20.5 mm; Nikon, Tokyo, Japan) and a charge-coupled device digital video camera system (SBC-320P B/W Camera; Samsung, Seoul, South Korea) connected to a video monitor (F—arteriole and G—capillary field [capillaries are marked by red arrows]). After *in vivo* staining of leukocytes with rhodamine 6G (Sigma-Aldrich, St. Louis, MO), leukocyte–endothelial interactions were assessed by intravital fluorescence microscopy (H; adhered leukocytes are marked by black arrows). (I) Schematic representation of the experimental protocol: after baseline determination of mean arterial blood pressure, heart rate, and microcirculatory parameters (t_0) animals received an intravenous injection of normal saline (NS) or lipopolysaccharide; sequential measurements of mean arterial blood pressure, heart rate, and microcirculatory parameters were performed at baseline (t_0), just before the initiation of a continuous intravenous infusion of NS or dexmedetomidine (t_1), and after 1 h (t_2), 2 h (t_3), and 3 h (t_4) of infusion (dotted lines). Rhodamine 6G injection for evaluation of leukocyte–endothelial interactions and blood sampling (black triangle) were performed after 3 h of NS or dexmedetomidine infusion; hamsters allocated in CONTROL group were submitted to a partial experimental protocol in which after baseline determination of mean arterial blood pressure and heart rate, animals bypassed NS or lipopolysaccharide injection and NS or dexmedetomidine infusion going directly to rhodamine 6G injection and blood sampling (black triangle). Photographs and photomicrographs are from an animal belonging to CONTROL group.

survived the entire experimental protocol leaving no missing data for statistical analysis.

Microcirculatory Parameters

At baseline, there were no significant differences in arteriolar and venular mean internal diameters and blood flow velocities between study groups. In venules, lipopolysaccharide elicited vasodilatation and decreased blood flow velocity. In arterioles, lipopolysaccharide induced moderate vasoconstriction and also decreased blood flow velocity. There were no significant differences in arteriolar and venular lipopolysaccharide responses between lipopolysaccharide–SALINE and lipopolysaccharide–DEXMEDETOMIDINE groups (table 1). At baseline, FCD and Erythrocyte-Vel did not significantly differ between study groups. In endotoxemic groups, FCD and Erythrocyte-Vel decreased after lipopolysaccharide administration. However, a major decrease was observed in lipopolysaccharide–SALINE group, which led to a significant difference between NS- and dexmedetomidine-treated endotoxemic animals. Thus, although significantly reduced when compared with nonendotoxemic animals, after lipopolysaccharide administration these variables exhibited significantly and maintained better temporal evolution in dexmedetomidine-treated animals than in those treated with NS (fig. 2).

Evaluation of Venular Leukocyte–Endothelial Interactions

The number of passing leukocytes decreased after endotoxemia induction ($P < 0.05$ vs. CONTROL or nonendotoxemic groups); statistically similar reductions were observed in lipopolysaccharide–SALINE and lipopolysaccharide–DEXMEDETOMIDINE groups (fig. 3). In endotoxemic animals, dexmedetomidine significantly reduced the number of rolling leukocytes to 49% of the value observed in

lipopolysaccharide–SALINE group (fig. 3). Endotoxemia significantly increased the number of adherent leukocytes in lipopolysaccharide–SALINE group, whereas there were no significant differences between CONTROL, SALINE–SALINE, SALINE–DEXMEDETOMIDINE, and lipopolysaccharide–DEXMEDETOMIDINE groups (fig. 3). Representative photomicrographs are shown in figure 4.

Correlations between Venular Leukocyte–Endothelial Interactions and Capillary Perfusion Changes

The number of passing leukocytes per minute was negatively correlated to the percentage reduction of FCD and Erythrocyte-Vel values during the experiment (from t0 to t4). On the other hand, the number of adherent leukocytes per square millimeters was positively correlated to the percentage reduction of these microcirculatory parameters. No other significant correlation was found (fig. 5).

MAP, HR, and Temperature Alterations

Mean arterial blood pressure and HR basal values were not significantly different among the experimental groups and were comparable to control values from healthy animals reported by the literature. Systemic administration of lipopolysaccharide elicited statistically similar reductions in MAP levels in lipopolysaccharide–SALINE and lipopolysaccharide–DEXMEDETOMIDINE groups (fig. 6). Lipopolysaccharide caused an initial increase (t1) followed by a significant decrease in HR (fig. 6). Dexmedetomidine induced a significant HR decrease in nonendotoxemic and endotoxemic animals, but a major decrease was observed in the endotoxemic group (lipopolysaccharide–DEXMEDETOMIDINE; fig. 6). Considering rectal and skinfold chamber temperatures, there were no significant differences between NS- and dexmedetomidine-treated animals.

Table 1. Arteriolar and Venular Mean Internal Diameters and Blood Flow Velocities

	Arteriolar Mean Internal Diameter (μm)	Arteriolar Blood Flow Velocity	Venular Mean Internal Diameter (μm)	Venular Blood Flow Velocity
SALINE–SALINE				
t0	66.3 \pm 12.0	3 (3/3)	72.1 \pm 15.6	3 (3/3.5)
t4	69.8 \pm 11.7*	3 (3/3)*	70.6 \pm 13.6	3 (3/3)
SALINE–DEXMEDETOMIDINE				
t0	61.9 \pm 7.4	3 (3/3)	66.1 \pm 11.9	3 (3/4)
t4	61.4 \pm 10.7	3 (3/3)	61.9 \pm 8.0*	3 (3/3)
Lipopolysaccharide–SALINE				
t0	66.7 \pm 11.6	3 (3/3)	69.9 \pm 10.1	4 (3/4)
t4	53.0 \pm 12.8†	2 (2/3)	83.0 \pm 12.5†	3 (2/3)‡
Lipopolysaccharide–DEXMEDETOMIDINE				
t0	66.8 \pm 13.0	3 (3/3)	75.2 \pm 11.8	4 (3/4)
t4	54.8 \pm 16.9†	2 (2/3)	82.1 \pm 13.2‡	2 (2/2.5)†§

Mean internal diameters are expressed as means \pm SD for each group. Blood flow velocities are expressed as median (Q1/Q3). t0, baseline; t4, 3h after normal saline (NS) or dexmedetomidine infusion.

* $P < 0.05$ as compared with endotoxemic groups at the same time point. † $P < 0.0001$ as compared with group baseline. ‡ $P < 0.05$ as compared with group baseline. § $P < 0.05$ as compared with nonendotoxemic groups at the same time point.

Lipopolysaccharide–DEXMEDETOMIDINE = endotoxemic, dexmedetomidine treated ($n = 12$); Lipopolysaccharide–SALINE = endotoxemic, NS treated ($n = 12$); SALINE–DEXMEDETOMIDINE = nonendotoxemic, dexmedetomidine treated ($n = 10$); SALINE–SALINE = nonendotoxemic, NS treated ($n = 10$).

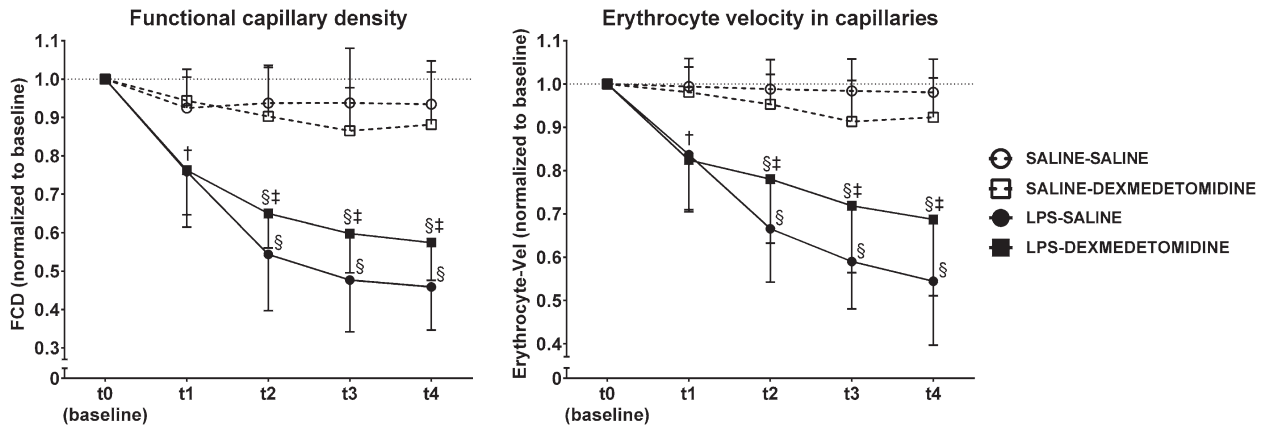


Fig. 2. Functional capillary density (FCD) and Erythrocyte-Vel evolution during the experimental period. Note that a significant difference was observed between lipopolysaccharide–SALINE and lipopolysaccharide–DEXMEDETOMIDINE groups, indicative of attenuation of lipopolysaccharide-induced capillary perfusion deficits associated with dexmedetomidine administration. FCD and Erythrocyte-Vel values (given as means \pm SD) were measured at baseline (t0), 1 h after normal saline or lipopolysaccharide administration (t1), and after 1 (t2), 2 (t3), and 3 (t4) hours of normal saline or dexmedetomidine infusion, and are presented as ratios relative to baseline values. SALINE–SALINE, nonendotoxemic, normal saline treated ($n = 10$); SALINE–DEXMEDETOMIDINE, nonendotoxemic, dexmedetomidine treated ($n = 10$); lipopolysaccharide–SALINE, endotoxemic, normal saline treated ($n = 12$); lipopolysaccharide–DEXMEDETOMIDINE, endotoxemic, dexmedetomidine treated ($n = 12$). † $P < 0.05$ as compared with nonendotoxemic groups at the same time point. § $P < 0.0001$ as compared with nonendotoxemic groups at the same time point. ‡ $P < 0.05$ as compared with lipopolysaccharide–SALINE group at the same time point.

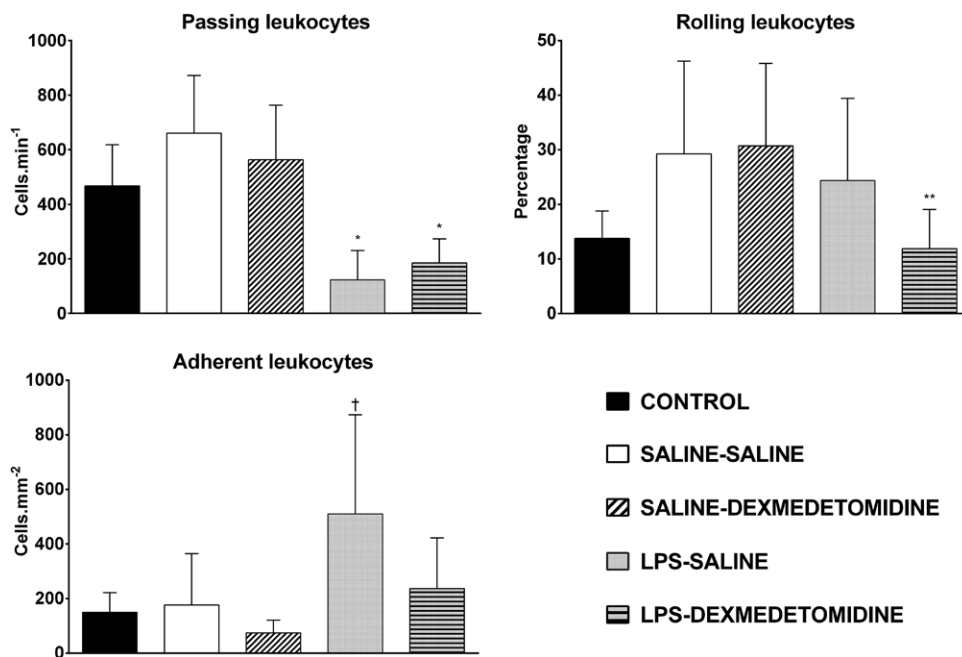


Fig. 3. Number of passing leukocytes per minute, percentage of rolling leukocytes, and number of adherent leukocytes per square millimeters on the hamster window chamber microcirculation. Data are given as means \pm SD. CONTROL, baseline controls ($n = 5$); SALINE–SALINE, nonendotoxemic, normal saline treated ($n = 10$); SALINE–DEXMEDETOMIDINE, nonendotoxemic, dexmedetomidine treated ($n = 10$); lipopolysaccharide–SALINE, endotoxemic, normal saline treated ($n = 12$); lipopolysaccharide–DEXMEDETOMIDINE, endotoxemic, dexmedetomidine treated ($n = 12$). * $P < 0.05$ as compared with CONTROL, SALINE–SALINE, or SALINE–DEXMEDETOMIDINE groups. ** $P < 0.05$ as compared with SALINE–SALINE, SALINE–DEXMEDETOMIDINE or lipopolysaccharide–SALINE groups. † $P < 0.05$ as compared with any other group.

Arterial Blood Gases and Lactate Analysis

A significant increase in arterial lactate concentration was observed in endotoxemic groups (lipopolysaccharide–SALINE and lipopolysaccharide–DEXMEDETOMIDINE);

however, there were no statistical differences between NS- and dexmedetomidine-treated groups (table 2). By contrast, a marked reduction in arterial HCO_3^- , BE, and TCO_2 was observed in lipopolysaccharide–SALINE group, which led

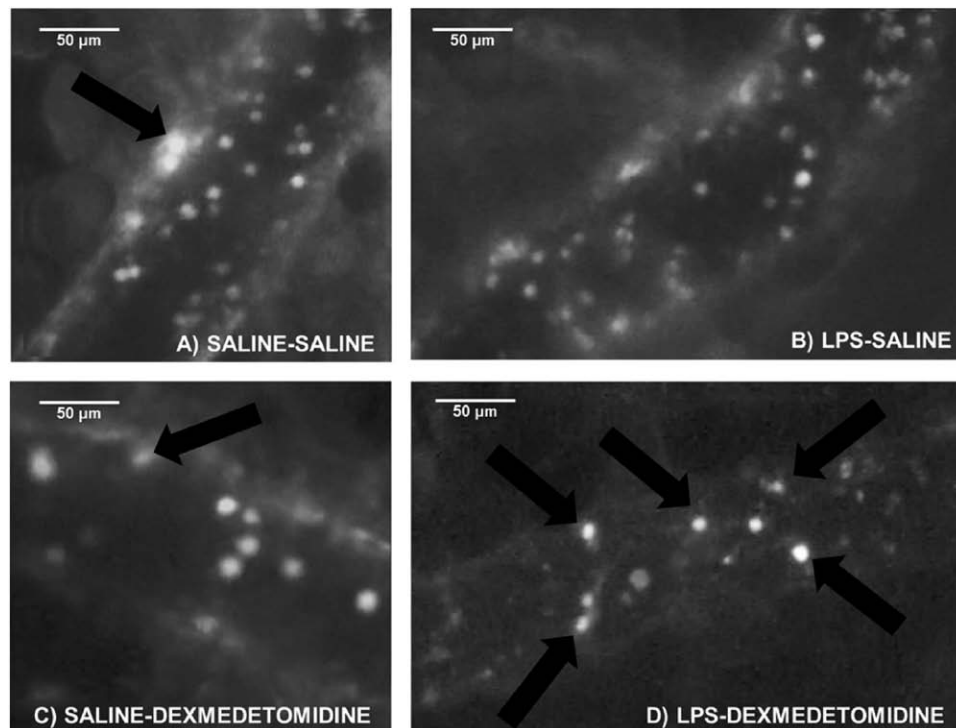


Fig. 4. Representative photomicrographs of the skinfold microcirculation after *in vivo* staining of leukocytes with rhodamine 6G. Intravital fluorescence microscopy of leukocytes labeled with fluorochrome rhodamine 6G allows the study of leukocyte–endothelial interactions in the microvasculature. Only a few adhered leukocytes (marked by *black arrows*) are normally observed in nonendotoxemic animals (*A* and *C*). Lipopolysaccharide induces a significant increase in venular leukocyte adherence. This was evidenced in *B* (lipopolysaccharide–SALINE group) where almost all observed leukocytes are adhered. The number of adherent leukocytes (marked by *black arrows*) observed in *D* (lipopolysaccharide–DEXMEDETOMIDINE group) was not statistically different than that observed in nonendotoxemic animals, showing an attenuation of the response to lipopolysaccharide with dexmedetomidine treatment.

to a significant difference between NS- and dexmedetomidine-treated endotoxemic animals (table 2). PO_2 increased after endotoxemia induction. PCO_2 was not significantly different between study groups.

Discussion

In our study, lipopolysaccharide administration increased venular leukocyte rolling and adhesion and decreased functional capillary density and erythrocyte velocity in the hamster skinfold chamber microcirculation, and induced metabolic acidosis, whereas dexmedetomidine treatment significantly attenuated these responses. Therefore, our key result was that dexmedetomidine treatment in the setting of experimental sepsis has partially restored microcirculatory function, improving tissue perfusion.

The lipopolysaccharide dose used in our study was adjusted to affect microcirculatory parameters without the induction of severe hypotension, whereas dexmedetomidine dose ($5.0 \mu\text{g kg}^{-1} \text{h}^{-1}$) was chosen based on previously published experimental studies that showed that this dose was able to reduce cytokine levels in endotoxemic rodents, increasing survival rate.^{17–19} Although $5.0 \mu\text{g kg}^{-1} \text{h}^{-1}$ is a high dose compared to that recommended for human use,

smaller animals tend to have higher drug needs in relation to their body mass due to their higher metabolic rates.²⁰ With this dose, animals achieved a mild sedation state, assessed by a murine sedation scale,²¹ and had a decrease in HR. MAP was not significantly affected by the infusion. MAP and HR evolution observed in our study is in agreement with the literature. Despite theoretical concerns regarding a possible circulatory worsening with dexmedetomidine use in septic patients, studies have shown that in critically ill patients both cardiac output and blood pressure are satisfactorily maintained during dexmedetomidine infusion, regardless of the fall in HR.^{6,22–25} Indeed, α -2 adrenergic receptor agonists may even restore pressor responsiveness to norepinephrine in experimental sepsis.⁷

In regard to the microcirculation, α -2 adrenergic receptor agonists have dual vasomotor effects: direct vasoconstriction (*via* “post”-synaptic α -1 and “extra”-synaptic α -2 adrenergic receptors when high plasma concentrations of dexmedetomidine are present, such as observed during bolus injection or fast infusion) and indirect vasodilatation (*via* central sympathetic inhibition, reduced peripheral release of norepinephrine, and nitric oxide dilation). Because nitric oxide deregulation and heterogeneity of production is one of the hallmarks of sepsis, it would be

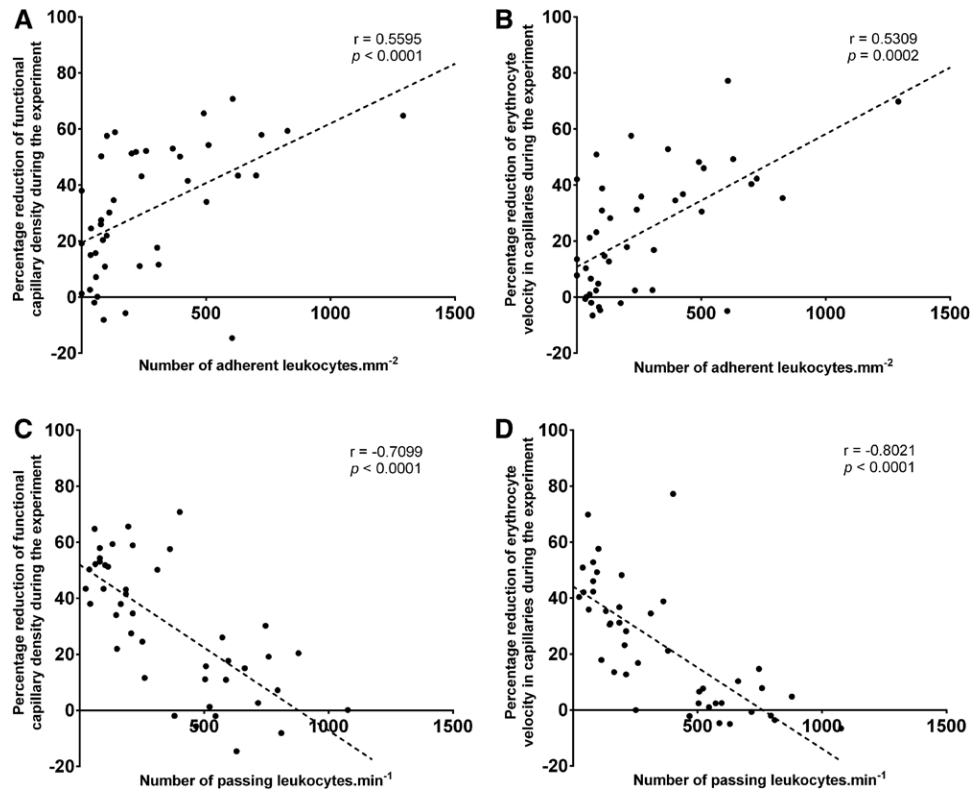


Fig. 5. Scatter plots of significant correlations between venular leukocyte–endothelial interactions and capillary perfusion changes. In A and B note that the less leukocytes adhere to the endothelium, the better the functional capillary density and erythrocyte velocity in capillaries are at the end of the experiment. By contrast, in C and D the more numerous the passing leukocytes per minute are, the better the microcirculation performs. Spearman correlation coefficient and *P* values are shown for each correlation ($n = 44$).

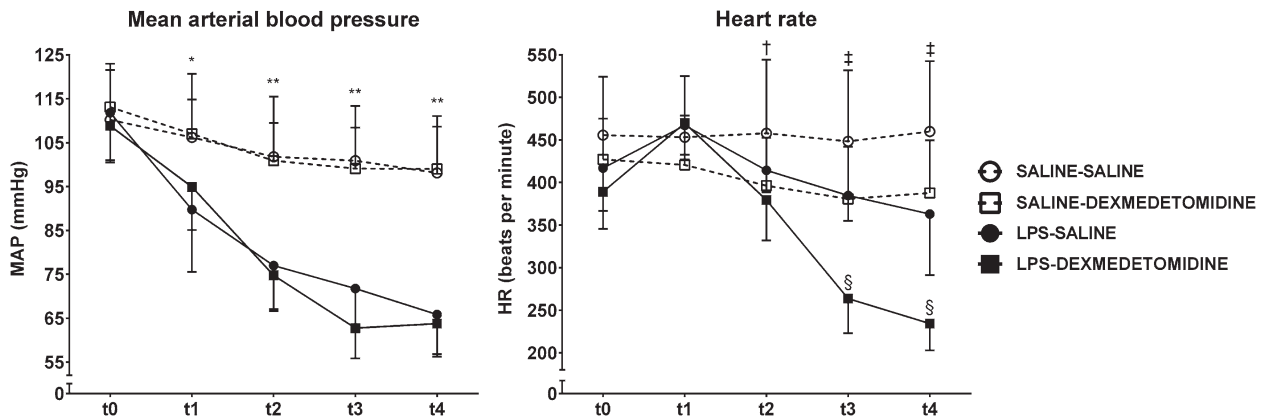


Fig. 6. Mean arterial blood pressure (MAP) and heart rate (HR) evolution during the experimental period. Values (given as means \pm SD) were measured at baseline (t0), 1 h after normal saline or lipopolysaccharide administration (t1), and after 1 h (t2), 2 h (t3), and 3 h (t4) of normal saline or dexmedetomidine infusion. SALINE–SALINE, nonendotoxemic, normal saline treated ($n = 10$); SALINE–DEXMEDETOMIDINE, nonendotoxemic, dexmedetomidine treated ($n = 10$); lipopolysaccharide–SALINE, endotoxemic, normal saline treated ($n = 12$); lipopolysaccharide–DEXMEDETOMIDINE, endotoxemic, dexmedetomidine treated ($n = 12$). * $P < 0.05$ as compared with endotoxemic groups at the same time point. ** $P < 0.0001$ as compared with endotoxemic groups at the same time point. † $P < 0.05$ as compared with lipopolysaccharide–DEXMEDETOMIDINE group at the same time point. ‡ $P < 0.05$ as compared with SALINE–DEXMEDETOMIDINE and lipopolysaccharide–SALINE groups at the same time point. § $P < 0.0001$ as compared with any other group at the same time point.

expected that the vasoconstrictor effect prevails, reducing tissue and organic perfusion.^{3,8,26,27} However, no significant differences were observed in arteriolar and venular mean internal diameters between lipopolysaccharide–SALINE

and lipopolysaccharide–DEXMEDETOMIDINE groups in our study.

Considering FCD and Erythrocyte-Vel temporal evolution, dexmedetomidine was associated with significant

Table 2. Arterial Blood Gases and Lactate

	Lactate (mmol/l)	pH	HCO ₃ (mmol/l)	BE (mmol/l)	TCO ₂ (mmol/l)	PCO ₂ (mmHg)	PO ₂ (mmHg)	SatO ₂ (%)
CONTROL	1.54±0.22	7.38±0.03	32.3±2.4	7.0±2.3	33.8±2.4	54.4±7.6	69.6±9.7	92.4±3.5
SALINE–SALINE	1.19±0.22	7.39±0.04	30.1±3.4	5.1±3.9	31.6±3.5	49.5±5.1	78.3±13.0	94.4±3.0
SALINE– DEXMEDETOMIDINE	1.36±0.57	7.40±0.04	30.4±1.4	5.6±1.5	31.9±1.7	48.6±5.5	94.2±20.9	96.6±1.4
Lipopolysaccharide– SALINE	2.99±0.72*	7.31±0.04†	24.4±2.1‡	−1.7±2.7‡	25.9±2.2‡	48.0±4.1	106.7±31.9§	95.8±3.6
Lipopolysaccharide– DEXMEDETOMIDINE	2.65±0.38*	7.33±0.10	29.0±2.4	3.2±3.1	30.8±2.5	55.1±14.1	131.0±24.8*	97.7±3.4§

Blood sampling for arterial blood gases and lactate analysis was performed at the end of the study period. Values are expressed as means ± SD for each group.

* $P < 0.05$ as compared with CONTROL, SALINE–SALINE, or SALINE–DEXMEDETOMIDINE groups. † $P < 0.05$ as compared with SALINE–SALINE or SALINE–DEXMEDETOMIDINE groups. ‡ $P < 0.05$ as compared with any other group. § $P < 0.05$ as compared with CONTROL or SALINE–SALINE.

CONTROL = baseline controls ($n = 5$); Lipopolysaccharide–DEXMEDETOMIDINE = endotoxemic, dexmedetomidine treated ($n = 12$); Lipopolysaccharide–SALINE = endotoxemic, NS treated ($n = 12$); NS = normal saline; SALINE–DEXMEDETOMIDINE = nonendotoxemic, dexmedetomidine treated ($n = 10$); SALINE–SALINE = nonendotoxemic, NS treated ($n = 10$).

attenuation of capillary perfusion deficits induced by lipopolysaccharide administration. Several factors are related to the microcirculatory impairment observed after endotoxemia induction, such as systemic hypotension and vasoconstriction.²⁸ Furthermore, lipopolysaccharide evokes leukocyte–endothelial interactions, resulting in capillary obstruction by leukocyte plugs and increased presence of rolling or adherent leukocytes in venules, which may hamper adequate capillary flow.²⁹ As better capillary perfusion in lipopolysaccharide–DEXMEDETOMIDINE group cannot be explained by MAP or HR changes or by changes in arteriolar or venular mean internal diameters or blood flow velocity, we speculate that the observed differences in leukocyte–endothelial interactions between lipopolysaccharide–SALINE and lipopolysaccharide–DEXMEDETOMIDINE groups have been crucial to our results. In fact, we could correlate venular leukocyte–endothelial interactions with capillary perfusion changes (fig. 5).

The decreased number of passing leukocytes observed after lipopolysaccharide administration is likely to be related to the activation and emigration of neutrophils out of the vascular bed and to sequestration of leukocytes in organs such as lungs and liver. This peripheral leukopenia becomes obvious 30 min after a single dose of lipopolysaccharide and persists for 8 h.³⁰ Of note, Uchiba *et al.*³¹ have correlated the accumulation of leukocytes in the lungs with pulmonary vascular injury induced by lipopolysaccharide. In a similar way, we have correlated a decreased number of passing leukocytes to a decreased capillary perfusion (fig. 5), suggesting that activation and sequestration of leukocytes contributes to the microvascular dysfunction observed after lipopolysaccharide administration. However, no significant differences were observed in the number of passing leukocytes between NS- and dexmedetomidine-treated endotoxemic animals.

Venular leukocyte rolling decreases 30 min after lipopolysaccharide (to about 10% of the value of passing leukocytes);

baseline conditions are reached again 3 h after lipopolysaccharide (when 20 to 30% of passing leukocytes are rolling) and an increase happens 8 h after lipopolysaccharide administration (to about 60% of the value of passing leukocytes).³⁰ Leukocyte–endothelial interactions were assessed 4 h after lipopolysaccharide administration allowing sufficient time for recovery of the basal number of rolling leukocytes (as observed in lipopolysaccharide–SALINE group). Dexmedetomidine treatment resulted in maintenance of a small percentage of rolling leukocytes in lipopolysaccharide–DEXMEDETOMIDINE group, denoting attenuation of the response to lipopolysaccharide (fig. 3). Finally, the number of adherent leukocytes did not increase in lipopolysaccharide–DEXMEDETOMIDINE group compared with nonendotoxemic animals, showing again attenuation of the response to lipopolysaccharide with dexmedetomidine (fig. 3). Interestingly, in our study we could correlate a smaller number of adherent leukocytes with a better capillary perfusion (fig. 5).

Of note, dexmedetomidine showed no effects on leukocyte–endothelial interactions in nonendotoxemic animals (SALINE–DEXMEDETOMIDINE group). It has already been shown that on proinflammatory environments, such as after surgery or injury, clonidine (another α -2 adrenergic receptor agonist) opposes leukocytes activation.^{32,33} This may explain why dexmedetomidine's effects on leukocyte–endothelial interactions were only seen after lipopolysaccharide injection.

Lower pH, HCO₃, BE, and TCO₂ values found in lipopolysaccharide–SALINE group are indicative of metabolic acidosis, probably secondary to tissue hypoperfusion. So, the absence of metabolic acidosis in lipopolysaccharide–DEXMEDETOMIDINE group suggests better tissue perfusion in this group compared with lipopolysaccharide–SALINE group, corroborating our microcirculatory findings. In two previous studies, Taniguchi *et al.*^{17,18} have already observed an attenuation in the degree of acidosis in

dexmedetomidine-treated endotoxemic rats when compared with endotoxemic controls. Since there is a latency between perfusion deficit correction and normalization of hyperlactatemia in septic patients, we hypothesize that the short treatment period with dexmedetomidine has been insufficient for a decrease in lactate concentrations in lipopolysaccharide-DEXMEDETOMIDINE group.³⁴ The contradictory PO₂ increase after lipopolysaccharide injection has been previously observed in endotoxemic hamsters and is likely to be related to an adaptive characteristic of the species to different oxygen consumption situations.³⁰

Perspectives and Limitations

Propofol, benzodiazepines, and dexmedetomidine are commonly used in intensive care units. Studies addressing the effects of propofol and midazolam on microcirculation agree that they exert negative effects, even in nonseptic states, leading to microcirculatory derangements.^{35,36} Conversely, we have shown that dexmedetomidine preserves microcirculatory parameters in nonendotoxemic animals and attenuates the effects of lipopolysaccharide on microcirculation. If these findings are replicated in humans, the clinical implication is that dexmedetomidine may improve microcirculatory function of septic patients, which is in line with many of the beneficial effects observed experimentally and clinically with dexmedetomidine use.

Our study has some limitations. First, even though lipopolysaccharide administration may reproduce many clinical features of sepsis syndrome it is in fact a model of experimental endotoxemia, so we cannot generalize/translate our results to the much more complex clinical scenario of human sepsis. Second, the absence of fluid resuscitation and nor-epinephrine infusion in our study further limits the generalizability of our results as both treatments are commonly used in human sepsis and have significant microcirculatory effects. In our study animals were not fluid resuscitated because this study was designed to evaluate the effects of dexmedetomidine on the microcirculation independently of fluid therapy effects. For a similar reason, we did not control lipopolysaccharide-induced changes in MAP with nor-epinephrine infusion (returning MAP to baseline values) in endotoxemic animals. Third, the study of the skin and subcutaneous muscle microcirculation may not be representative of microcirculatory changes in organs of crucial importance in the pathophysiology of sepsis, such as splanchnic organs, limiting our conclusions. However, the hamster skinfold window chamber model is widely used for microvascular studies in endotoxemic animals because the first reactions after lipopolysaccharide administration seem to be comparable in different tissues and organs.^{29,30,37,38} Furthermore, unlike other microcirculatory models, this one permits the existence of a recovery period between surgical manipulation for chamber implantation and the actual experiments, allowing recovery of microcirculatory function affected by surgical trauma. In addition, experiments can be performed

without background anesthesia, which has microcirculatory effects of its own.³⁹ Fourth, we did not attempt to delineate a specific dexmedetomidine action *via* α -2 adrenergic receptor (effect reversal with idazoxan) or *via* imidazoline receptors (effect reversal with efaroxan). Finally, central sympatholytic actions of dexmedetomidine could potentiate the cholinergic antiinflammatory pathway.^{9,40,41} Indeed, several studies have shown that dexmedetomidine reduces plasma concentration of proinflammatory cytokines in septic animals and humans.^{12,17–19,42} Since we did not quantitate these cytokines (lack of standardized laboratory tests for golden Syrian hamsters), we cannot make correlations between our leukocyte–endothelial interactions results and the inflammatory response.

Conclusions

In our study, dexmedetomidine administration was effective in reducing lipopolysaccharide-induced leukocyte–endothelial interactions in the hamster skinfold chamber microcirculation. This was accompanied by a significant attenuation of capillary perfusion deficits. These results suggest that dexmedetomidine yields beneficial effects on endotoxemic animals' microcirculation. Further studies in experimental models closer to human sepsis are required to confirm this benefit.

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Competing Interests

The authors declare no competing interests.

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BioVasc, Pavilhão Reitor Haroldo Lisboa da Cunha, Rio de Janeiro State University, Rua São Francisco Xavier 524, 20550-013 Rio de Janeiro, RJ, Brazil. marcosmiranda@gmail.com. Information on purchasing reprints may be found at www.anesthesiology.org or on the masthead page at the beginning of this issue. ANESTHESIOLOGY's articles are made freely accessible to all readers, for personal use only, 6 months from the cover date of the issue.

References

- Angus DC, Linde-Zwirble WT, Lidicker J, Clermont G, Carcillo J, Pinsky MR: Epidemiology of severe sepsis in the United States: Analysis of incidence, outcome, and associated costs of care. *Crit Care Med* 2001; 29:1303–10
- Martin GS, Mannino DM, Eaton S, Moss M: The epidemiology of sepsis in the United States from 1979 through 2000. *N Engl J Med* 2003; 348:1546–54
- Trzeciak S, Cinel I, Phillip Dellinger R, Shapiro NI, Arnold RC, Parrillo JE, Hollenberg SM; Microcirculatory Alterations in Resuscitation and Shock (MARS) Investigators: Resuscitating the microcirculation in sepsis: The central role of nitric oxide, emerging concepts for novel therapies, and challenges for clinical trials. *Acad Emerg Med* 2008; 15:399–413
- Pichot C, Ghignone M, Quintin L: Dexmedetomidine and clonidine: From second- to first-line sedative agents in the critical care setting? *J Intensive Care Med* 2012; 27: 219–37
- Venn RM, Hell J, Grounds RM: Respiratory effects of dexmedetomidine in the surgical patient requiring intensive care. *Crit Care* 2000; 4:302–8
- Pandharipande PP, Sanders RD, Girard TD, McGrane S, Thompson JL, Shintani AK, Herr DL, Maze M, Ely EW; MENDS Investigators: Effect of dexmedetomidine *versus* lorazepam on outcome in patients with sepsis: An a priori-designed analysis of the MENDS randomized controlled trial. *Crit Care* 2010; 14:R38
- 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:e431–8
- Iida H, Iida M, Ohata H, Nagase K, Dohi S: Hypothermia attenuates the vasodilator effects of dexmedetomidine on pial vessels in rabbits *in vivo*. *Anesth Analg* 2004; 98: 477–82
- Sanders RD, Hussell T, Maze M: Sedation & immunomodulation. *Anesthesiol Clin* 2011; 29:687–706
- Martins CR, Tardelli MA, Amaral JL: [Effects of dexmedetomidine on blood coagulation evaluated by thromboelastography]. *Rev Bras Anestesiol* 2003; 53:705–19
- Yeh YC, Sun WZ, Ko WJ, Chan WS, Fan SZ, Tsai JC, Lin TY: Dexmedetomidine prevents alterations of intestinal microcirculation that are induced by surgical stress and pain in a novel rat model. *Anesth Analg* 2012; 115:46–53
- 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; 98:550–2
- Committee for the Update of the Guide for the Care and Use of Laboratory Animals: Guide for the Care and Use of Laboratory Animals, 8th edition. Washington, D.C., National Academies Press, 2011, pp 1–220
- Endrich B, Asaishi K, Götz A, Messmer K: Technical report—A new chamber technique for microvascular studies in unanesthetized hamsters. *Res Exp Med (Berl)* 1980; 177:125–34
- Klyszcz T, Jünger M, Jung F, Zeintl H: [Cap image—A new kind of computer-assisted video image analysis system for dynamic capillary microscopy]. *Biomed Tech (Berl)* 1997; 42:168–75
- Dubin A, Pozo MO, Ferrara G, Murias G, Martins E, Canullán C, Canales HS, Kanoore Edul VS, Estenssoro E, Ince C: Systemic and microcirculatory responses to progressive hemorrhage. *Intensive Care Med* 2009; 35:556–64
- 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; 32:1322–6
- 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
- Qiao H, Sanders RD, Ma D, Wu X, Maze M: Sedation improves early outcome in severely septic Sprague Dawley rats. *Crit Care* 2009; 13:R136
- Sharma V, McNeill JH: To scale or not to scale: The principles of dose extrapolation. *Br J Pharmacol* 2009; 157:907–21
- Chuck TL, McLaughlin PJ, Arizzi-LaFrance MN, Salamone JD, Correa M: Comparison between multiple behavioral effects of peripheral ethanol administration in rats: Sedation, ataxia, and bradykinesia. *Life Sci* 2006; 79:154–61
- Venn M, Newman J, Grounds M: A phase II study to evaluate the efficacy of dexmedetomidine for sedation in the medical intensive care unit. *Intensive Care Med* 2003; 29:201–7
- Riker RR, Shehabi Y, Bokesch PM, Ceraso D, Wisemandle W, Koura F, Whitten P, Margolis BD, Byrne DW, Ely EW, Rocha MG; SEDCOM (Safety and Efficacy of Dexmedetomidine Compared With Midazolam) Study Group: Dexmedetomidine vs midazolam for sedation of critically ill patients: A randomized trial. *JAMA* 2009; 301:489–99
- Pandharipande PP, Pun BT, Herr DL, Maze M, Girard TD, Miller RR, Shintani AK, Thompson JL, Jackson JC, Deppen SA, Stiles RA, Dittus RS, Bernard GR, Ely EW: Effect of sedation with dexmedetomidine *vs* lorazepam on acute brain dysfunction in mechanically ventilated patients: The MENDS randomized controlled trial. *JAMA* 2007; 298:2644–53
- Tan JA, Ho KM: Use of dexmedetomidine as a sedative and analgesic agent in critically ill adult patients: A meta-analysis. *Intensive Care Med* 2010; 36:926–39
- Kirkeboen KA, Strand OA: The role of nitric oxide in sepsis—An overview. *Acta Anaesthesiol Scand* 1999; 43:275–88
- Dobrucki LW, Cabrera CL, Bohr DF, Malinski T: Central hypotensive action of clonidine requires nitric oxide. *Circulation* 2001; 104:1884–6
- Santos AO, Furtado ES, Villela NR, Bouskela E: Microcirculatory effects of fluid therapy and dopamine, associated or not to fluid therapy, in endotoxemic hamsters. *Clin Hemorheol Microcirc* 2011; 47:1–13
- Hoffmann JN, Vollmar B, Laschke MW, Inthorn D, Fertmann J, Schildberg FW, Menger MD: Microhemodynamic and cellular mechanisms of activated protein C action during endotoxemia. *Crit Care Med* 2004; 32:1011–7
- Hoffmann JN, Vollmar B, Inthorn D, Schildberg FW, Menger MD: A chronic model for intravital microscopic study of microcirculatory disorders and leukocyte/endothelial cell interaction during normotensive endotoxemia. *Shock* 1999; 12:355–64
- Uchiba M, Okajima K, Murakami K, Okabe H, Takatsuki K: Attenuation of endotoxin-induced pulmonary vascular injury by antithrombin III. *Am J Physiol* 1996; 270(6 Pt 1): 1921–30
- Sud R, Spengler RN, Nader ND, Ignatowski TA: Antinociception occurs with a reversal in alpha 2-adrenoceptor regulation of TNF production by peripheral monocytes/macrophages from pro- to anti-inflammatory. *Eur J Pharmacol* 2008; 588:217–31
- von Dossow V, Baehr N, Moshirzadeh M, von Heymann C, Braun JP, Hein OV, Sander M, Wernecke KD, Konertz W, Spies CD: Clonidine attenuated early proinflammatory response in T-cell subsets after cardiac surgery. *Anesth Analg* 2006; 103:809–14

34. Marik PE, Baram M: Noninvasive hemodynamic monitoring in the intensive care unit. *Crit Care Clin* 2007; 23:383–400
35. Koch M, De Backer D, Vincent JL, Barvais L, Hennart D, Schmartz D: Effects of propofol on human microcirculation. *Br J Anaesth* 2008; 101:473–8
36. Lamblin V, Favory R, Boulo M, Mathieu D: Microcirculatory alterations induced by sedation in intensive care patients. Effects of midazolam alone and in association with sufentanil. *Crit Care* 2006; 10:R176
37. Hoffmann JN, Vollmar B, Inthorn D, Schildberg FW, Menger MD: The thrombin antagonist hirudin fails to inhibit endotoxin-induced leukocyte/endothelial cell interaction and microvascular perfusion failure. *Shock* 2000; 14:528–34
38. Hoffmann JN, Vollmar B, Laschke MW, Inthorn D, Schildberg FW, Menger MD: Hydroxyethyl starch (130 kD), but not crystalloid volume support, improves microcirculation during normotensive endotoxemia. *ANESTHESIOLOGY* 2002; 97:460–70
39. Brookes ZL, Brown NJ, Reilly CS: Intravenous anaesthesia and the rat microcirculation: The dorsal microcirculatory chamber. *Br J Anaesth* 2000; 85:901–3
40. Tracey KJ: Physiology and immunology of the cholinergic anti-inflammatory pathway. *J Clin Invest* 2007; 117:289–96
41. Martelli D, McKinley MJ, McAllen RM: The cholinergic anti-inflammatory pathway: A critical review. *Auton Neurosci* 2014; 182:65–9
42. Hofer S, Steppan J, Wagner T, Funke B, Lichtenstern C, Martin E, Graf BM, Bierhaus A, Weigand MA: Central sympatholytics prolong survival in experimental sepsis. *Crit Care* 2009; 13:R11