Effects of Dexmedetomidine on Intestinal Microcirculation and Intestinal Epithelial Barrier in Endotoxemic Rats

Yu-Chang Yeh, M.D., Ph.D., Chun-Yu Wu, M.D., Ya-Jung Cheng, M.D., Ph.D., Chih-Min Liu, M.D., Jong-Kai Hsiao, M.D., Ph.D., Wing-Sum Chan, M.D., Zong-Gin Wu, B.S., Linda Chia-Hui Yu, Ph.D., Wei-Zen Sun, M.D.

ABSTRACT

Background: Dexmedetomidine reduces cytokine production in septic patients and reduces inflammation and mortality in experimental models of endotoxemia and sepsis. This study investigated whether dexmedetomidine attenuates endothelial dysfunction, intestinal microcirculatory dysfunction, and intestinal epithelial barrier disruption in endotoxemic rats.

Methods: Ninety-two male Wistar rats were randomly assigned to the following four groups: (1) Sham; (2) lipopolysaccharide, received IV lipopolysaccharide 15 and 10 mg/kg at 0 and 120 min; (3) dexmedetomidine, received IV dexmedetomidine for 240 min; and (4) lipopolysaccharide + dexmedetomidine, received both lipopolysaccharide and dexmedetomidine. Sidestream dark-field videomicroscope, tissue oxygen monitor, and full-field laser perfusion image were used to investigate the microcirculation of the terminal ileum. Serum endocan level was measured. The Ussing chamber permeability assay, lumen-to-blood gadodiamide passage by magnetic resonance imaging, and bacterial translocation were conducted to determine epithelial barrier function. Mucosal apoptotic levels and tight junctional integrity were also examined.

Results: The density of perfused small vessels in mucosa, serosal muscular layer, and Peyer patch in the lipopolysaccharide + dexmedetomidine group was higher than that of the lipopolysaccharide group. Serum endocan level was lower in the lipopolysaccharide + dexmedetomidine group than in the lipopolysaccharide group. Mucosal ratio of cleaved to full-length occludin and spleen bacterial counts were significantly lower in the lipopolysaccharide + dexmedetomidine group than in the lipopolysaccharide group.

Conclusion: The study finding suggests that dexmedetomidine protects against intestinal epithelial barrier disruption in endotoxemic rats by attenuating intestinal microcirculatory dysfunction and reducing mucosal cell death and tight junctional damage. **(Anesthesiology 2016; 125:355-67)**

DEXMEDETOMIDINE is a potent α2-adrenoceptor agonist[1] and widely used sedative in mechanically ventilated patients in intensive care units.[2] Pandharipande *et al.*[3] reported that the duration of mechanical ventilation was shorter and the mortality was lower in septic patients receiving dexmedetomidine than in those receiving a lorazepam-based sedation regimen. Although it was an *a priori*–designed subgroup analysis of the Maximizing Efficacy of Targeted Sedation and Reducing Neurological Dysfunction trial,[4] the authors suggested that their findings might have resulted from the antiinflammatory effects and organ-protective properties of dexmedetomidine.[5,6] Moreover, Memiş *et al.*[7] reported that dexmedetomidine infusion reduces cytokine production in critically ill septic patients. Dexmedetomidine has been reported to enhance macrophagic activity[8] and reduce inflammation and mortality in endotoxemia and polymicrobial sepsis animal models in several studies.[9–11]

What We Already Know about This Topic

- Dexmedetomidine reduces inflammation and cytokine production in experimental models of sepsis, but its effects on the integrity and microcirculatory function of the intestinal barrier remain unknown

What This Article Tells Us That Is New

- Using a multifaceted experimental approach in endotoxemic rats, the authors found dexmedetomidine to protect against intestinal epithelial barrier disruption by attenuating intestinal microcirculatory dysfunction and reducing mucosal cell death and tight junctional damage

Several studies have revealed sepsis-related microcirculatory dysfunction, namely, decreased perfused vessel density,[12] microthrombosis,[13] shunting,[14] endothelial dysfunction, and capillary hyperpermeability.[15] Severe microcirculatory

Drs. Sun and Yu contributed equally to the research project.

Submitted for publication September 21, 2015. Accepted for publication March 23, 2016. From the Department of Anesthesiology, National Taiwan University Hospital (Y.-C.Y., C.-Y.W., Y.-J.C., C.-M.L., Z.-G.W., W.-Z.S.) and the Graduate Institutes of Physiology, College of Medicine (L.C.-H.Y.), National Taiwan University, Taipei, Taiwan, Republic of China; Department of Medical Imaging, Taipei Buddhist Tzu Chi General Hospital, New Taipei, Taiwan, Republic of China (J.-K.H.); and Department of Anesthesiology, Far Eastern Memorial Hospital, New Taipei, Taiwan, Republic of China (W.-S.C.).

 Anesthesiology 2016; 125:355-67

<zdoi;10.1097/ALN.0000000000001135>

dysfunction and ischemic injury in the highly vascularized intestine may cause enteric bacterial translocation and systemic inflammatory responses, eventually leading to multiple organ dysfunction and death.[16,17] Endothelial cells play a key role in sepsis pathogenesis and microcirculatory dysfunction,[18–20] and an endothelial cell–specific molecule-1 (endocan, a 50-kDa dermatan sulfate proteoglycan) is expressed on endothelial cells and is acknowledged as a suitable marker of endothelial dysfunction.[21,22] Furthermore, Frey and Kesel[23] reported that splanchnic perfusion decreases early in sepsis. In our previous study, dexmedetomidine attenuated intestinal microcirculation alteration in a rat model of surgical stress and pain stimulation.[24] In the current study, we aimed to test the first hypothesis that the antiinflammatory effect of dexmedetomidine can alleviate intestinal microcirculatory dysfunction and reduces endothelial dysfunction in endotoxemic rats. Moreover, intestinal mucosal ischemia can cause mucosal cell death–dependent barrier disruption and subsequent bacterial translocation,[25–27] and some investigators have suggested that the gut is a source of multiple organ failure.[28,29] Dexmedetomidine administration before ischemia can attenuate intestinal injury induced by intestinal ischemia–reperfusion in rats.[30] Furthermore, perioperative administration of dexmedetomidine has been shown to attenuate intestinal injury in patients.[31] Our previous studies have shown that epithelial cell death is partly responsible for tight junctional damage and bacterial translocation.[32,33] We speculate that if intestinal microcirculation could be preserved, epithelial cell death due to ischemia might also be prevented. Therefore, we aimed to test the second hypothesis that restoration of intestinal microcirculation may reduce the severity of epithelial cell death and intestinal barrier damage.

The primary objective of this study was to determine whether dexmedetomidine can reduce intestinal microcirculatory and endothelial dysfunction in endotoxemic rats by using a sidestream dark-field (SDF) videomicroscope, a tissue oxygen monitor, a full-field laser perfusion imager,[34,35] and an endocan assay kit. The secondary objective of this study was to investigate the integrity of the intestinal barrier by using the Ussing chamber permeability assay and lumen-to-blood passage of gadodiamide by magnetic resonance imaging (MRI).[36] In addition, epithelial cell death, tight junctional damage, and bacterial translocation to extraintestinal organs were investigated.

Materials and Methods

A total of 92 male Wistar rats (BioLASCO Taiwan Co., Taiwan) weighing 250 ± 50 g were used in this study, which was approved by the Animal Care and Use Committee of Laboratory Animal Center (No. 20130367; College of Medicine, National Taiwan University, Taipei, Taiwan, Republic of China). The rats were maintained on a 12-h light–dark cycle and had *ad libitum* access to water and food. The study was divided into three parts. Part A was designed to investigate the effects of dexmedetomidine on the microcirculation of the terminal ileum in endotoxemic rats and to determine the biomarkers of endothelial dysfunction; 40 rats were used. Part B was designed to determine the effect of dexmedetomidine on the intestinal epithelial permeability by *ex vivo* assay, bacterial translocation from the gut lumen to the liver and spleen; 40 rats were used. Part C was designed to investigate the effect of dexmedetomidine on the intestinal epithelial permeability by *in vivo* assay, tight junctional damage, and mucosal cell death levels; 12 rats were used. Considering the high variation of the severity of sepsis in cecal ligation and puncture model, we chose endotoxemic model for this experiment based on our experience from a previous study.

Grouping and Treatment Protocol

In parts A and B, the rats were randomly assigned to the following four groups (fig. 1). (1) Rats receiving 1.5 and 1 ml/kg 0.9% saline intravenously at 0 and 120 min, respectively, comprised the Sham group. Saline (0.9%) was continuously infused at 2 ml · kg^{-1} · h^{-1} as a maintenance fluid supplement through the external jugular vein catheter by using an infusion pump (KDS101; KD Scientific, USA). (2) Rats receiving 15 and 10 mg/kg lipopolysaccharide (*Escherichia coli*, serotype O127:B8; Sigma, USA; 10 mg/ml in 0.9% saline) intravenously at 0 and 120 min, respectively, and continuous IV infusion of 0.9% saline at 2 ml · kg^{-1} · h^{-1} comprised the lipopolysaccharide group. (3) Rats receiving 1.5 and 1 ml/kg 0.9% saline intravenously at 0 and 120 min, respectively, and continuous IV infusion of dexmedetomidine (Precedex; Hospira, USA) at 5 μg · kg^{-1} · h^{-1} in 0.9% saline at 2 ml · kg^{-1} · h^{-1} from 0 to 240 min comprised the dexmedetomidine group. (4) Rats receiving 15 and 10 mg/kg lipopolysaccharide intravenously at 0 and 120 min, respectively, and continuous IV infusion of dexmedetomidine at 5 μg · kg^{-1} · h^{-1} in 0.9% saline at 2 ml · kg^{-1} · h^{-1} from 0 to 240 min comprised the lipopolysaccharide + dexmedetomidine group. The dosage of dexmedetomidine was determined by our pilot study and the studies by Taniguchi *et al.*[9] and Zhang *et al.*[30] In part C, the rats were randomly assigned to the following three groups: the Sham group, the lipopolysaccharide group, and the lipopolysaccharide + dexmedetomidine group.

Part A: Effects on Terminal Ileum Microcirculation

Anesthesia and Surgical Procedure. Initial anesthesia, weighing, and shaving of abdominal hair were performed as described in our previous study.[35] Atropine sulfate (0.05 mg/kg in 5 ml/kg 0.9% saline; Taiwan Biotech Co., Ltd., Taiwan) was administered subcutaneously. A 14-gauge catheter was inserted into the trachea *via* tracheostomy and connected to the anesthesia machine. The rats spontaneously breathed, and subsequent anesthesia was maintained with 1.0% isoflurane in oxygen (0.6 l/min). Polyethylene catheters were inserted into the right common carotid artery and external jugular vein. Arterial blood pressure and heart rate were continuously monitored using Biopac Student Lab

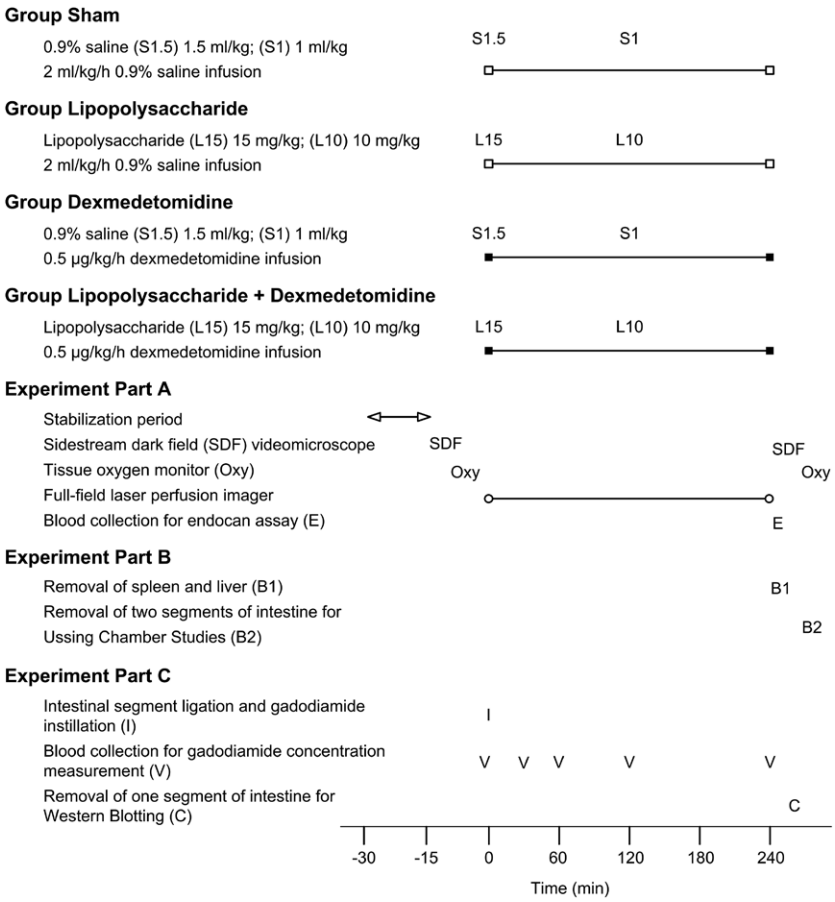

Fig. 1. Grouping and treatment protocol.

PRO 3.7 software and MP45 hardware (BIOPAC Systems Inc., USA). Rectal temperature was continuously monitored. After the abdominal wall was disinfected, a 3-cm-long midline laparotomy was performed using sterile instruments. After locating the cecum, a segment of the terminal ileum (approximately 6 to 10 cm proximal to the ileocecal valve) was carefully exteriorized, and the mesenteric arteries were examined to avoid stretching or kinking. A 2-cm-long incision was made at the antimesenteric site of the intestinal lumen by using a high-frequency desiccator (Aaron 900; Bovie Medical, USA). Three intestinal sites (the mucosa, serosal muscular layer, and Peyer patch) were located to examine the microcirculation. The rats were observed for a 15-min equilibration period.[35]

After 15 min, the microcirculation in the 40 rats of the four groups was examined using SDF videomicroscope (MicroScan; MicroVision Medical, The Netherlands) on the mucosa, serosal muscular layer, and Peyer patch. A tissue oxygen monitor (moorVMS-OXY; Moor Instruments, United Kingdom) was used for measuring the relative concentration of oxygenated hemoglobin (oxyHb), relative concentration of deoxygenated hemoglobin (deoxyHb), and tissue oxygen saturation (StO_2) of the mucosa, serosal muscular layer, and Peyer patch. After the examinations, the time point of the experiment was set as 0 min, and the rats received treatment according to their grouping and treatment protocol. The mucosa, serosal muscular layer, and Peyer patch of the rats were continuously examined using a full-field laser perfusion imager (moorFLPI; Moor Instruments Ltd.) for 240 min. At the end of the experiment, SDF videomicroscopy and tissue oxygen monitoring were performed again, and blood was withdrawn from the right common carotid artery catheter for laboratory analysis. The rats were euthanized through exsanguination under anesthesia.

SDF Videomicroscopy Examinations. The total small vessel (less than 20 μm) density, blood flow classification of small vessels, perfused small vessel density (PSVD), proportion of perfused small vessels (PPV), microvascular flow index (MFI) score, and heterogeneity index were investigated according to the suggestion of the round table conference for evaluating microcirculation.[37] The blood flow of each small vessel was classified by a semiquantitative method as follows: (0) absent (either no flow or filled with microthrombosis), (1) intermittent flow (presence of flow for less than 50% of the observation time), (2) sluggish flow, and (3) continuous flow.[37] The setting of videomicroscope was performed as described in our previous study.[35] Three continuous 10-s image sequences were recorded for each measured site. The images were analyzed using automated analysis software (AVA 3.0; Academic Medical Center, University of Amsterdam, Amsterdam, The

Netherlands), and data on the three images were averaged for statistical analysis. The measurement and calculation of these microcirculation parameter were performed as described in our previous study.[35] The analyses were performed by a single investigator who was blinded to the grouping.

Tissue Oxygen Monitor Examinations. The moorVMS-OXY operates on the basis of the theory of white light reflectance spectroscopy. Measurements using this instrument rely on spectrophotometric principles that associate light absorption with chromophore concentrations. Measurements are performed using probes, which are placed in contact with the tissue at the measurement site. Optical fibers are used to deliver illumination light to the tissue and collect reflectance light for processing, and analysis is performed using spectra in the visible wavelength range between 500 and 650 nm. OxyHb is displayed as an arbitrary unit. The StO_2 reading is displayed as a percentage of the oxyHb divided by the relative total hemoglobin concentration (total Hb = oxyHb + deoxyHb).

Full-field Laser Perfusion Imager Examinations. The intensity of microvascular blood flow was measured using a full-field laser perfusion imager by employing the laser speckle contrast imaging technique, as described in our previous study.[34] Three regions of interest (ROIs) were set at the mode of single-point measurement: ROI 1 was selected on the mucosa, ROI 2 was selected on the serosal muscular layer, and ROI 3 was selected on the Peyer patch. The microvascular blood flow intensities of each ROI were recorded as flux with the perfusion unit. The changes of microvascular blood flow intensity were expressed as percentage changes of blood flux from the baseline (0 min) and were subsequently used for statistical analysis.

Examination of Serum Endocan Level. Serum endocan levels were measured using the Rat-Endocan ELISA Kit (Lunginnov SAS, France) according to the manufacturer's instructions.

Part B: Effects on Intestinal Epithelial Permeability and Bacterial Translocation to Extraintestinal Organs

Anesthesia and Surgical Procedure for Ussing Chamber Studies and Bacterial Translocation to Extraintestinal Organs. Rats were anesthetized with urethane (1.2 g/kg in 5 ml/kg 0.9% saline, intraperitoneal injection; Sigma), and 0.05 mg/kg atropine sulfate in 5 ml/kg 0.9% saline was administered subcutaneously. Tracheostomy was performed, and the rats spontaneously breathed. A polyethylene catheter was inserted into the external jugular vein for subsequent IV treatments. The rats were observed for a 15-min equilibration period, and the time point of the experiment was set as 0 min. The rats received treatment according to their grouping and treatment protocol. After 240 min, aseptic laparotomy was performed through a midline incision by using sterile instruments. The spleen and liver were removed from the rats, and the tissue weights were determined. After locating the cecum, two 3-cm segments of the terminal ileum (approximately 6 to 9 cm and 12 to 15 cm proximal to the ileocecal valve) were carefully removed, and the luminal contents were gently rinsed out using phosphate-buffered saline.

Ussing Chamber Studies. Muscle-stripped intestinal segments (2 cm) were opened along the mesenteric border and mounted on Ussing chambers (World Precision Instruments, USA). The chamber was prepared as described in our previous study.[36,38] A circulating water bath maintained the buffer temperature at 37°C. The potential difference (mV) was measured, and the short-circuit current (Isc, $\mu A/cm^2$) of the tissue was determined in a voltage-clamp mode on line. The tissue was subjected to 1-mV, 1-s pulses in 5-min intervals, and the Isc change caused by the pulses was used for calculating the intestinal tissue conductance (mS/cm^2) according to Ohm law.[39] A greater conductance indicated higher intestinal permeability. The level of mucosal-to-serosal flux of dextran conjugated to fluorescein isothiocyanate (FITC) (dextran-FITC, molecular weight [MW] = 4 kDa; Sigma) was used to determine intestinal epithelial permeability.[40] Tissues mounted on the Ussing chambers were allowed to equilibrate until the Isc stabilized, before the dextran probe was added to the mucosal buffer at a final concentration of 500 μM. Serosal buffer samples (250 μl) were collected at 0, 30, 60, 90, and 120 min after placement of dextran probe and were replaced with Krebs buffer. A multimode plate reader was used to determine the fluorescence units of dextran-FITC at excitation/emission = 490/530 nm, and the concentration (nM) was calculated.[41] The mucosal-to-serosal flux levels of dextran-FITC at 30, 60, 90, and 120 min minus the baseline level (0 min) were expressed as an indicator of the intestinal epithelial permeability.

Analysis of Intestinal Bacterial Translocation to Extraintestinal Organs. The tissues were homogenized and sonicated in sterile phosphate-buffered saline with a ratio of 1 mg to 10 μl.[42] Undiluted lysate (200 μl) was inoculated onto fresh blood agar plates (Scientific Biotech Corp., Taiwan). After incubation at 37°C for 24 h, the number of bacterial colonies was enumerated and normalized to colony-forming units per gram of tissue.[38]

Part C: Effects on In Vivo *Intestinal Epithelial Permeability, Tight Junctional Damage, and Mucosal Cell Death Levels*

MRI-based Gut Permeability Assay. Initial anesthesia and operation were performed as described in our previous study.[35] To evaluate the changes in intestinal permeability *in vivo* and the lumen-to-blood passage of a small molecule, a novel MRI-based assay was conducted by applying a contrast probe gadodiamide (Gadoscan, MW = 574 Da; InnoPharmax, Taiwan) to the lumen of a ligated jejunal sac immediately after saline or lipopolysaccharide injection. After opening the abdominal wall, an 8-cm jejunal sac was created starting 6 cm proximal to the ileocecal valve for probe injection. Gadodiamide was instilled into the lumen of ligated jejunal sac at 0.25 M in 1 ml volume immediately after saline or lipopolysaccharide injection. The blood samples were collected before (0 min) or after injection at 30, 60, 120, and

240 min. For measurement of serum gadodiamide concentration, a standard solution with known concentration (0.5 M) of gadodiamide was diluted by a two-fold series ranging from 2^{-18} to 2^{-29} M with 0.9% saline. Each serum sample and each standard solution were, respectively, collected in a 200-μl polypropylene tube. All samples were assayed in duplicates. We used 1.5-Tesla MRI (GE Signa Excite; General Electric, USA) for measuring the signal intensity of the serum. These test tubes were placed in a 96-well rack in a homemade water tank and then subjected to MRI scan. Images were obtained in the transverse and axial plane of the entire tube using 1.5-mm-thick slices. We selected Fast spin echo with T1-weighted sequence for imaging. Imaging parameters were as follows: repetition time = 400 ms, echo time = 11.8 ms, field of view = 14 × 14 cm, and number of excitations = 6. Matrix size was 256 × 192, and the number of slice was 10. The total scan time was 20 min.

The signal intensity of each sample solution was measured by circling a region of 4 mm^2 in MRI image using the workstation provided by GE Healthcare (United Kingdom). The signal intensity of serum samples was divided by that of background noise and expressed as signal-to-noise ratio. The signal-to-noise ratio of the standard solutions of gadodiamide was plotted against the concentration thereof to establish a standard curve. The gadodiamide concentration in serum samples at each time point was calculated by using the standard curve.

Western Blotting for Tight Junctional Damage and Mucosal Cell Death Levels. Intestinal mucosal proteins were extracted with complete radioimmunoprecipitation buffer and subjected to sodium dodecyl sulfate/polyacrylamide gel electrophoresis (4 to 13% polyacrylamide) as described.[42–44] The resolved proteins were then electrotransferred onto polyvinylidene difluoride or nitrocellulose membranes in a semidry blotter. Blots were blocked with 5% (w/v) nonfat dry milk in Tris-buffered saline (TBS) or 5% (w/v) bovine serum albumin in TBS with Tween-20 (TBS-T; 0.1% [v/v] Tween-20 in TBS) for 1 h, washed with TBS-T, and incubated with a primary antibody at 4°C overnight. The membrane was washed and incubated with a secondary antibody for 1 h. After washing, the membranes were incubated with chemiluminescent solution and signals detected. The primary antibodies used included anti-occludin (1:1,000; Invitrogen, USA), anti-caspase-3 (1:2,000; Cell Signaling Technology, USA), anticleaved caspase-3 (1:2,000; Cell Signaling Technology), and anti-β-actin (1:10,000; Sigma). The secondary antibodies used were horseradish peroxidase–conjugated goat anti-mouse immunoglobulin G (1:2,000; Santa Cruz, USA) and anti-rabbit immunoglobulin G (1:1,000; Cell Signaling Technology).

Statistical Analysis

Data were analyzed using statistical software (SPSS 20; IBM SPSS, USA). Based on previous experience, 10 rats per group were needed for testing the primary hypothesis regarding the changes of microvascular blood flow intensity. Continuous variables were presented as mean (SD). Mean differences at different time points were investigated using two-way repeated-measures ANOVA (with time and group factors), followed by the Tukey test for between-groups multiple comparison. Serum endocan levels and intestinal mucosa conductance were analyzed using one-way ANOVA followed by *post hoc* analysis using the Tukey test. All tests were two tailed. A P value less than 0.05 was considered significant. MFI scores and bacterial colony counts were presented as the median (interquartile range) and analyzed using the Mann–Whitney U test. While comparing bacterial colony counts, we defined three comparison pairs as follows: Sham *versus* lipopolysaccharide, Sham *versus* lipopolysaccharide + dexmedetomidine, and lipopolysaccharide *versus* lipopolysaccharide + dexmedetomidine. The Bonferroni correction was used, and a P value less than 0.017 was considered significant.

Results

Systemic Hemodynamics and Body Temperature

The heart rate was significantly increased in the lipopolysaccharide rats compared with the Sham controls, and dexmedetomidine attenuated lipopolysaccharide-induced tachycardia (table 1). The mean arterial pressure of endotoxemic rats was significantly lower than that of the Sham controls. No significant difference was observed between the lipopolysaccharide and lipopolysaccharide + dexmedetomidine groups (table 1), indicating that dexmedetomidine did not potentiate endotoxemia-related hypotension. The body temperature of endotoxemic rats was significantly higher than that of the Sham controls, and no significant difference was observed between the lipopolysaccharide and lipopolysaccharide + dexmedetomidine groups (table 1).

Microcirculation of Terminal Ileum and Endocan Levels

Absence of blood flow or microthrombosis in small vessels was observed in endotoxemic rats (fig. 2), and it resulted in lower PSVD and PPV. The PSVD and PPV of the mucosa, serosal muscular layer, and Peyer patch in the lipopolysaccharide group were significantly lower than those in the Sham and lipopolysaccharide + dexmedetomidine groups (table 2). The heterogeneity index of the mucosa was higher in the lipopolysaccharide group than in the lipopolysaccharide + dexmedetomidine group. At 240 min, the MFI scores of the lipopolysaccharide group were significantly lower than those of the lipopolysaccharide + dexmedetomidine group at the mucosa and serosal muscular layer, but not at the Peyer patch (table 2). The StO_2 values of the mucosa and Peyer patch in the lipopolysaccharide group were significantly lower than those in the Sham and lipopolysaccharide + dexmedetomidine groups; however, the difference in StO_2 value at the serosal muscular layer was not significant between

Table 1. Mean Arterial Pressure, Heart Rates, and Body Temperature over Time

Group	0 Min	60 Min	120 Min	180 Min	240 Min	*P* Value*		
						Time	Group	Interaction
Mean arterial pressure (mmHg)						< 0.001	0.013	< 0.001
Sham	102 (10)	102 (10)	103 (8)	105 (6)	103 (8)			
Lipopolysaccharide	101 (8)	103 (8)	103 (7)	101 (9)	79 (20)†			
Dexmedetomidine	106 (7)	96 (14)	92 (12)	94 (9)†	90 (13)			
Lipopolysaccharide + Dexmedetomidine	101 (8)	101 (7)	100 (13)	92 (11)†	79 (8)†			
Heart rate (beats/min)						< 0.001	< 0.001	< 0.001
Sham	399 (27)	414 (28)	414 (31)	420 (34)	429 (46)			
Lipopolysaccharide	379 (28)	435 (37)‡	459 (43)†‡	486 (41)†‡	503 (44)†‡			
Dexmedetomidine	381 (38)	354 (49)†	331 (27)†	336 (17)†	340 (18)†			
Lipopolysaccharide + dexmedetomidine	389 (31)	381 (32)	387 (41)	408 (46)	454 (51)			
Body temperature (°C)						< 0.001	< 0.001	< 0.001
Sham	36.7 (0.5)	37.5 (0.3)	37.4 (0.3)†	37.3 (0.3)	37.4 (0.4)			
Lipopolysaccharide	37.0 (0.4)	38.2 (0.7)†	38.7 (0.7)†	39.3 (0.8)†	40.0 (1.1)†			
Dexmedetomidine	36.8 (0.4)	37.2 (0.5)	37.2 (0.3)	37.2 (0.5)	36.9 (0.5)			
Lipopolysaccharide + dexmedetomidine	37.1 (0.5)	38.4 (0.4)†	38.9 (0.8)†	39.5 (1.1)†	39.8 (1.5)†			

Data are represented as the mean (SD), n = 10 for each group.

*By repeated ANOVA measurements. †$P < 0.05$ *vs*. the sham group using one-way ANOVA with Tukey test. ‡$P < 0.05$ *vs*. the lipopolysaccharide + dexmedetomidine group using one-way ANOVA with Tukey test.

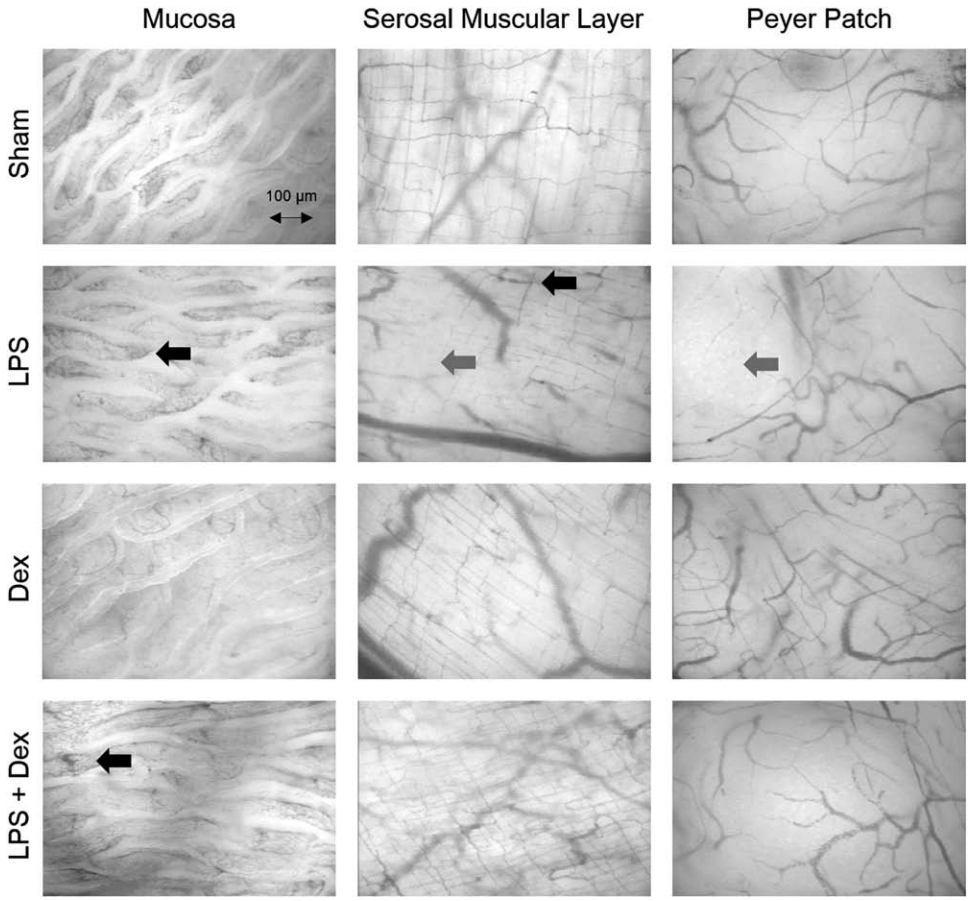

Fig. 2. Terminal ileum microcirculation images obtained using sidestream dark-field videomicroscopy at 240 min. Absence of flow or filled with microthrombosis in small vessels (*black arrow*). Intermittent blood flow in some small vessels (*gray arrow*). Dex = dexmedetomidine; LPS = lipopolysaccharide.

Table 2. Comparison of Terminal Ileum Microcirculation at 0 and 240 Min

Group	TSVD 0 Min	TSVD 240 Min	PSVD 0 Min	PSVD 240 Min	PPV 0 Min	PPV 240 Min	MFI 240 Min	HI 240 Min
Mucosa								
Sham	29.5 (1.9)	28.3 (2.3)	29.5 (1.9)	28.2 (2.3)	100 (0)	100 (0)	3 (3–3)	0 (0)
Lipopolysaccharide	28.8 (2.0)	28.5 (2.6)	38.8 (2.0)	8.6 (8.4)*†	100 (0)	31 (31)*†	0.9 (0.5–1.5)‡	1.24 (0.95)§‖
Dexmedetomidine	29.7 (1.7)	28.7 (1.7)	29.7 (1.7)	28.6 (1.6)	100 (0)	100 (0)	3 (3–3)	0 (0)
Lipopolysaccharide + Dexmedetomidine	28.5 (3.1)	27.7 (1.9)	28.5 (3.1)	24.6 (6.2)	100 (0)	89 (22)	3 (2.7–3)	0.12 (0.21)
Serosal muscular layer								
Sham	29.0 (3.2)	28.3 (4.1)	28.8 (3.0)	28.3 (4.1)	100 (1)	100 (0)	3 (3–3)	0 (0)
Lipopolysaccharide	26.3 (2.4)	29.6 (2.5)	26.3 (2.4)	11.6 (9.7)*†	100 (0)	40 (34)*†	1.3 (0.5–2.6)‡	0.50 (0.64)§
Dexmedetomidine	29.0 (2.6)	28.3 (1.7)	28.9 (2.7)	28.2 (1.8)	100 (1)	100 (1)	3 (3–3)	0 (0)
Lipopolysaccharide + Dexmedetomidine	29.3 (1.4)	31.6 (2.6)	29.2 (1.4)	28.1 (7.8)	100 (0)	89 (22)	3 (2.3–3)	0.18 (0.30)
Peyer patch								
Sham	27.1 (1.6)	27.3 (1.9)	26.5 (1.4)	27.1 (1.8)†	98 (2)	99 (1)†	3 (3–3)	0 (0)
Lipopolysaccharide	26.0 (1.9)	27.5 (2.3)	24.3 (1.4)	9.8 (7.9)*†	94 (7)	36 (30)*†	1.4 (0.7–1.9)	0.98 (0.70)§
Dexmedetomidine	25.7 (2.6)	27.0 (1.2)	25.0 (2.6)	25.9 (1.3)†	97 (2)	96 (3)†	3 (3–3)	0 (0)
Lipopolysaccharide + dexmedetomidine	27.2 (1.7)	28.1 (1.9)	25.7 (1.5)	18.3 (8.0)*	95 (5)	64 (26)*	2.4 (1.3–3)	0.50 (0.57)

Data are represented as the mean (SD) or median (interquartile range), n = 10 for each group.

*P < 0.05 *vs.* the Sham group using two-way ANOVA with the Tukey test. †P < 0.05 *vs.* the lipopolysaccharide + dexmedetomidine group using two-way ANOVA with the Tukey test. ‡P < 0.01 *vs.* the lipopolysaccharide + dexmedetomidine group using the Mann–Whitney U test. §P < 0.05 *vs.* the Sham group using one-way ANOVA with the Tukey test. ‖P < 0.05 *vs.* the lipopolysaccharide + dexmedetomidine group using one-way ANOVA with the Tukey test.

HI = heterogeneity index; MFI = microvascular flow index; PPV = proportion of perfused small vessels; PSVD = perfused small vessel density; TSVD = total small vessel density.

the lipopolysaccharide and lipopolysaccharide + dexmedetomidine groups (table 3). At 240 min, the percentage changes of blood flux from the baseline were higher in the lipopolysaccharide group than in the lipopolysaccharide + dexmedetomidine group (mucosa: −33[16]% *vs.* −11[9]%, P = 0.001; serosal muscular layer: −16[10]% *vs.* −2[9]%, P = 0.021; and Peyer patch: −40[13]% *vs.* −20[11]%, P = 0.011) (fig. 3). The serum endocan levels of the lipopolysaccharide group were higher than those of the Sham and lipopolysaccharide + dexmedetomidine groups (table 4).

Intestinal Epithelial Permeability, Tight Junctional Integrity, and Bacterial Translocation to Extraintestinal Organs

The intestinal tissue conductance of the lipopolysaccharide group was higher than that of the Sham controls, whereas the conductance values of the lipopolysaccharide + dexmedetomidine group were not significantly different from that of the Sham controls (table 4). The levels of mucosal-to-serosal flux of dextran-FITC (MW = 3,000 Da) were higher in the lipopolysaccharide group than in the Sham controls (fig. 4). However, the dextran flux was not reduced in the lipopolysaccharide + dexmedetomidine group (fig. 4). The plasma gadodiamide concentration was eight times higher in the lipopolysaccharide group (0.134 ± 0.112 μM) compared to sham group (0.016 ± 0.003 μM) after 240 min but did not reach statistical significance. A trend of decrease was in the plasma gadodiamide concentration of lipopolysaccharide + dexmedetomidine group (0.034 ± 0.010 μM) compared to lipopolysaccharide group (fig. 5). We next investigated alteration of tight junction molecules in rat intestinal mucosa. Western blotting results showed a significant reduction of the ratio of cleaved to full-length occludin in the lipopolysaccharide + dexmedetomidine group compared to the lipopolysaccharide group, suggesting amelioration of tight junctional damage (fig. 6). Lastly, the numbers of liver and spleen bacterial colonies of endotoxemic rats were statistically higher than those of Sham controls. Moreover, the number of spleen bacterial colonies in the lipopolysaccharide + dexmedetomidine group was significantly decreased compared to the lipopolysaccharide group (table 4).

Cell Death Level of Intestinal Mucosa

The cell death levels in rat intestinal mucosa were determined by Western blots of cleaved caspase-3 (a hallmark of cell apoptosis). The ratio of cleaved to full-length caspase-3 was statistically higher in the lipopolysaccharide group than in the Sham controls (fig. 6). The ratio of cleaved to full-length caspase-3 in the lipopolysaccharide + dexmedetomidine group was not significantly different from that of Sham controls.

Discussion

In this study, we provide evidence that dexmedetomidine can improve intestinal microcirculatory dysfunction, reduce

Table 3. Comparison of Tissue Oxygenated Hemoglobin Concentration and Oxygen Saturation of the Terminal Ileum at 0 and 240 Min

AU	OxyHb	OxyHb	Total Hb	Total Hb	StO_2 (%)	StO_2 (%)
Group	0 min	240 min	0 min	240 min	0 min	240 min
Mucosa						
Sham	134 (28)	114 (49)	206 (39)	181 (56)	65 (3)	60 (6)
Lipopolysaccharide	123 (33)	54 (37)*†	190 (41)	150 (37)†	64 (4)	30 (17)*†
Dexmedetomidine	114 (18)	70 (15)†	180 (29)	140 (20)†	63 (3)	49 (5)
Lipopolysaccharide + dexmedetomidine	131 (20)	116 (37)	205 (32)	211 (57)	64 (3)	54 (8)
Serosal muscular layer						
Sham	152 (27)	158 (48)	203 (37)	226 (62)	74 (2)	68 (5)
Lipopolysaccharide	161 (34)	124 (56)	216 (40)	243 (58)	73 (4)	47 (14)*
Dexmedetomidine	176 (36)	128 (46)	233 (43)	195 (53)	75 (4)	64 (7)
Lipopolysaccharide + dexmedetomidine	170 (55)	157 (42)	230 (70)	263 (39)	73 (3)	59 (11)
Peyer patch						
Sham	153 (33)	117 (30)	213 (38)	176 (39)	70 (4)	65 (4)
Lipopolysaccharide	140 (36)	95 (43)	200 (42)	214 (46)	69 (4)	44 (17)*†
Dexmedetomidine	143 (36)	110 (33)	206 (43)	174 (32)	68 (5)	61 (6)
Lipopolysaccharide + dexmedetomidine	145 (77)	124 (49)	236 (83)	223 (47)	70 (7)	60 (5)

Data are represented as the mean (SD), n = 10 for each group.

*P < 0.05 *vs.* the sham group using two-way ANOVA with the Tukey test. †P < 0.05 *vs.* the lipopolysaccharide + dexmedetomidine group using two-way ANOVA with the Tukey test.

AU = arbitrary unit; OxyHb = oxygenated hemoglobin; StO_2 = oxyHb divided by total Hb; Total Hb = OxyHb + deoxygenated hemoglobin.

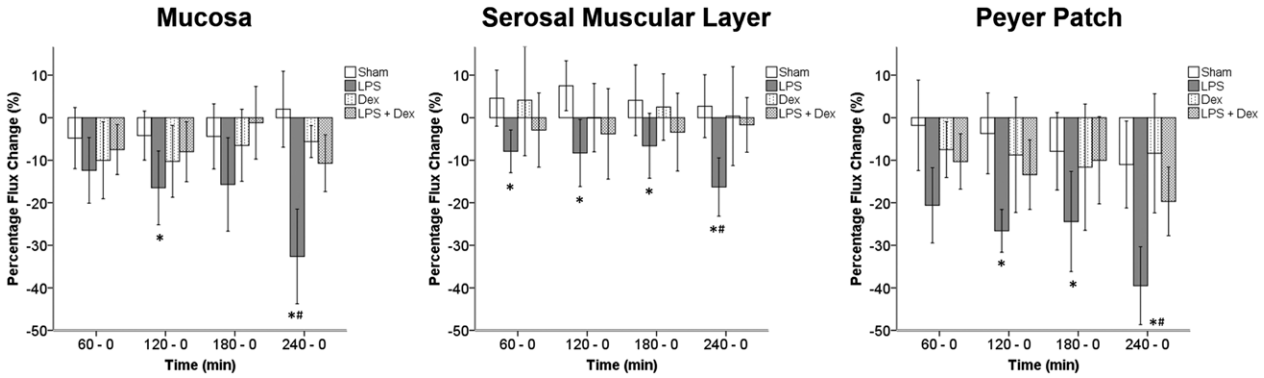

Fig. 3. Percentage changes of flux at each time point compared with 0 min at three measured sites of the terminal ileum. The *error bars* represent the 95% CIs of the means. A two-way repeated-measures ANOVA with the Tukey test for between-groups multiple comparison was used for statistical analysis. Flux represents microvascular blood flow intensity. *P < 0.05 *versus* the Sham group. #P < 0.05 *versus* the lipopolysaccharide (LPS) + dexmedetomidine (Dex) group.

endothelial dysfunction and excessive tachycardia, and attenuate intestinal epithelial cell death, tight junction damage, and intestinal bacterial translocation to the spleen in endotoxemic rats. Administering dexmedetomidine alleviates endotoxemia-related intestinal microcirculatory dysfunction, as evidenced by the higher PSVD values, blood flux, and MFI scores. In addition, dexmedetomidine reduced serum endocan level, suggesting its protective action against endothelial dysfunction. Moreover, our data demonstrated that lipopolysaccharide-induced mucosal cell apoptosis was attenuated by dexmedetomidine in rats, in association with reduction of occludin cleavage and bacterial influx into spleen. These findings suggest that dexmedetomidine, by improving microcirculation, may attenuate lipopolysaccharide-induced epithelial cell death as a means to protect against gut barrier dysfunction. The hypothesis and results of this study are summarized in figure 7.

Two major mechanisms support the finding that dexmedetomidine preserves the microcirculation in endotoxemic rats. First, the percentage changes of blood flux from the baseline at the three measured sites were lower in the lipopolysaccharide + dexmedetomidine group than in the lipopolysaccharide group. It evidences that the microcirculatory blood flow was higher in the lipopolysaccharide

Table 4. Comparison of Serum Endocan Level, Intestinal Tissue Conductance, and Bacterial Translocation to Extraintestinal Organs

Group	Endocan (ng/ml)	Conductance (mS/cm^2)	Bacterial Colonies (CFU/g)	
			Liver	Spleen
Sham	0.20 (0.10)	28 (9)	0 (0–75)	0 (0–0)
Lipopolysaccharide	0.70 (0.33)*†	45 (16)*	250 (150–400)‡	400 (150–800)‡§
Dexmedetomidine	0.22 (0.06)	29 (9)	0 (0–0)	0 (0–0)
Lipopolysaccharide + dexmedetomidine	0.33 (0.08)	37 (10)	300 (188–500)‡	175 (94–300)‡

Data are represented as mean (SD) or median (interquartile range), n = 10 for each group. Two intestinal segments of each rats were examined for conductance.
*P < 0.05 *vs.* the Sham group using one-way ANOVA with the Tukey test. †P < 0.05 *vs.* the lipopolysaccharide + dexmedetomidine group using one-way ANOVA with the Tukey test. ‡P < 0.017 *vs.* the Sam group using the Mann–Whitney U test. §P < 0.017 *vs.* the lipopolysaccharide + dexmedetomidine group using the Mann–Whitney U test.
CFU = colony-forming units; Endocan = endothelial cell–specific molecule-1.

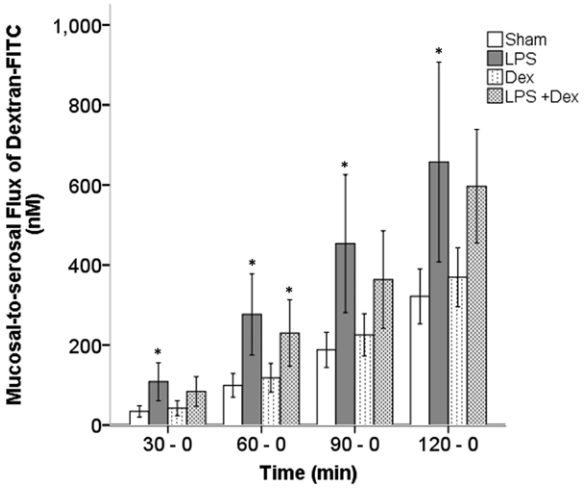

Fig. 4. Mucosal-to-serosal flux levels of dextran–fluorescein isothiocyanate (FITC) at each time point compared with 0 min. Dextran-FITC, dextran conjugated to FITC. A two-way repeated-measures ANOVA with the Tukey test for between-groups multiple comparison was used for statistical analysis. The P values were as follows: time factor, P < 0.001; time-group interaction, P = 0.004; between-groups effect, P = 0.001. The *error bars* represent the 95% CIs of the means. *P < 0.05 *versus* the Sham group. Dex = dexmedetomidine; LPS = lipopolysaccharide.

+ dexmedetomidine group than in the lipopolysaccharide group. Second, PSVD and PPV were lower in the lipopolysaccharide group than in the lipopolysaccharide + dexmedetomidine group. It represented that more microthrombosis formation, absence of blood flow, or intermittent flow was noted in the small vessels of the lipopolysaccharide group. Excessive inflammation,[45,46] leukocyte adherence,[47–49] and endothelial cell injury[50,51] alter coagulation and fibrinolysis during endotoxemia or sepsis, and these alterations may cause disseminated intravascular coagulation. The antiinflammatory effects of dexmedetomidine were proven by several studies[5,7,9–11]; moreover, Miranda *et al.*[52] demonstrated that dexmedetomidine reduces lipopolysaccharide-induced leukocyte adherence and leukocyte–endothelial interactions. Furthermore, in our study, the lower serum endocan level in the lipopolysaccharide + dexmedetomidine group than in the lipopolysaccharide group suggests that dexmedetomidine may reduce the severity of endothelial dysfunction in endotoxemic rats.

We found for the first time that dexmedetomidine can attenuate lipopolysaccharide-induced epithelial cell death as a means to protect against gut barrier dysfunction. The evidence is that the ratio of cleaved to full-length caspase-3 was higher in the lipopolysaccharide group than in the Sham controls. The ratio in the lipopolysaccharide + dexmedetomidine group was not significantly different from that of Sham controls. Moreover, there was a significant reduction in the ratio of cleaved to full-length occludin in the lipopolysaccharide + dexmedetomidine group compared to the lipopolysaccharide group, suggesting amelioration of tight junctional damage. The lipopolysaccharide-induced enteric bacterial translocation to spleen suggested impaired epithelial barrier integrity, which was reduced by dexmedetomidine. However, dexmedetomidine did not reduce the liver bacterial counts. We suspect that aside from limiting enteric bacterial translocation, dexmedetomidine might also reduce microbial counts in the bloodstream and spleen through enhancing microcirculation and infiltration of phagocytes to facilitate in bacterial clearance.[53]

Bradycardia is a commonly reported adverse event occurring during dexmedetomidine administration, and there is a crucial concern that dexmedetomidine may impede compensated tachycardia and reduce cardiac output. We suggest that adequate tachycardia may increase cardiac output during early sepsis; nevertheless, excessive tachycardia may result in an opposite response. In our study, the antisympathetic effect of dexmedetomidine significantly attenuated excessive tachycardia, and microvascular blood flow was well preserved. Furthermore, Morelli *et al.*[54] reported that heart rate control using a titrated esmolol (a cardioselective β1-receptor blocker) infusion in septic shock patients was associated with the maintenance of stroke volume and preservation of microvascular blood flow. In addition to bradycardia, hypotension is another antisympathetic effect of dexmedetomidine and is the most commonly reported adverse event in critically ill patients.[55,56] In nonshock critically ill patients, hypotension is usually prevented by omitting the loading dose or resolved without intervention.[2,57]

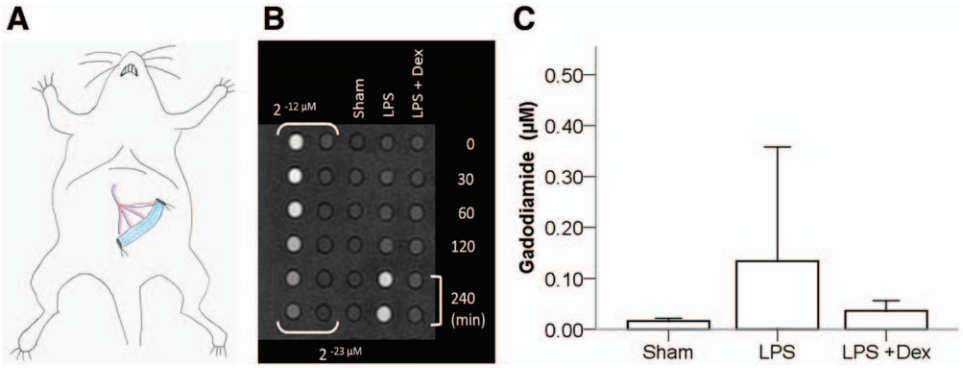

Fig. 5. Lumen-to-blood passage of a small molecule by a magnetic resonance imaging–based assay. (*A*) Schematic illustration of a contrast agent, gadodiamide (molecular weight = 574 Da), instilled into the ligated jejunal sac. (*B*) Representative magnetic resonance images of rat plasma samples and standard curve of known concentration of gadodiamide in plates. *Left*: Standard concentration of gadodiamide prepared in two-fold serial dilution. *Right*: Plasma samples from three rat groups. (*C*) Quantification of the plasma concentration of gadodiamide in rats after 240 min as an index of gut permeability. The *error bars* represent 2 SEM. Dex = dexmedetomidine; LPS = lipopolysaccharide.

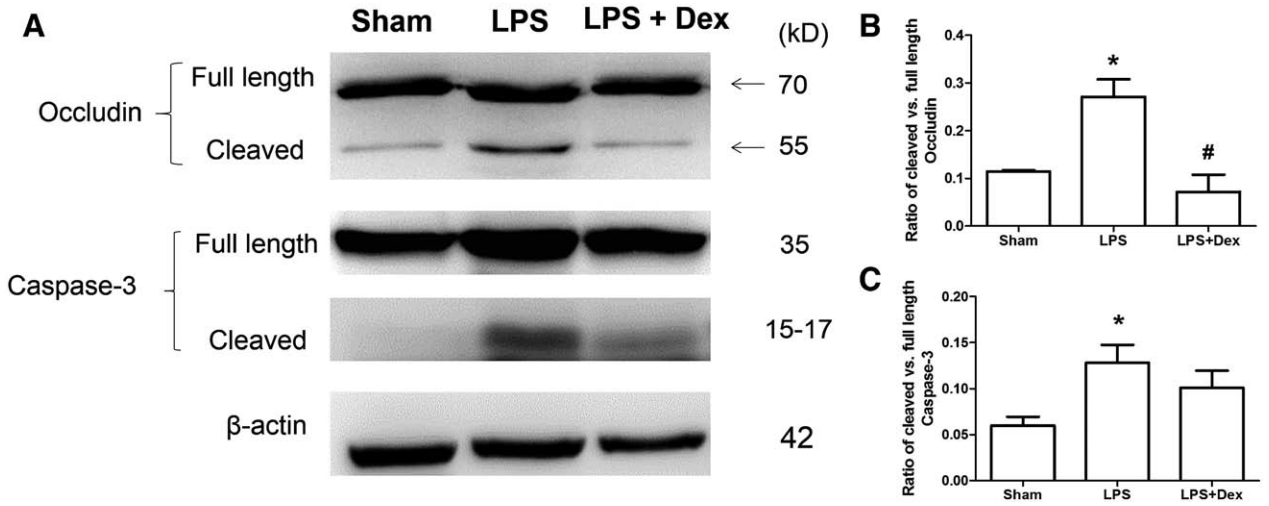

Fig. 6. Dexmedetomidine (Dex) treatment reduces tight junctional destruction and decreases mucosal apoptosis in lipopolysaccharide (LPS) groups. (*A*) Western blots showing full-length and cleaved occludin, as well as full-length and cleaved caspase-3 levels in rat intestinal mucosa. (*B*) Densitometric analysis of ratio of cleaved to full-length occludin. (*C*) Densitometric analysis of ratio of cleaved to full-length caspase-3. The *error bars* represent 2 SEM. *$P < 0.05$ *versus* the Sham group. #$P < 0.05$ *versus* the LPS group.

In septic shock patients, hypotension is usually prevented by omitting the loading dose, providing an adequate fluid supplement, or coadministering vasopressors. Another common clinical concern is that dexmedetomidine may potentiate the severity of hypotension. Our results suggest that the mean arterial pressure was not significantly different between endotoxemic rats with and those without dexmedetomidine infusion. Another serious concern is that dexmedetomidine may increase the infusion doses of vasopressors. Geloen *et al.*[58] demonstrated that dexmedetomidine increases the vasopressor response to norepinephrine in endotoxemic rats. To investigate the clinical effect of dexmedetomidine in sepsis patients, a multicenter clinical trial investigating 28-day mortality is ongoing in Japan (ClinicalTrials.gov Identifier: NCT01760967). Moreover, we are conducting a randomized controlled trial to investigate the effect of dexmedetomidine on sublingual microcirculation in severe sepsis patients (ClinicalTrials.gov Identifier: NCT02109965).

There are several limitations in this study. First, the results of this study are limited to the endotoxemic model. The effects of dexmedetomidine in the sepsis model require further investigation. Second, the observation time of this endotoxemic model is short. The effects of dexmedetomidine after 240 min require further investigation. Third, the investigation of intestinal microcirculation is focused on the terminal ileum. Further research is required to determine the effects of dexmedetomidine on the microcirculation of other regions of intestine. Fourth, the arterial and central venous blood gas analyses

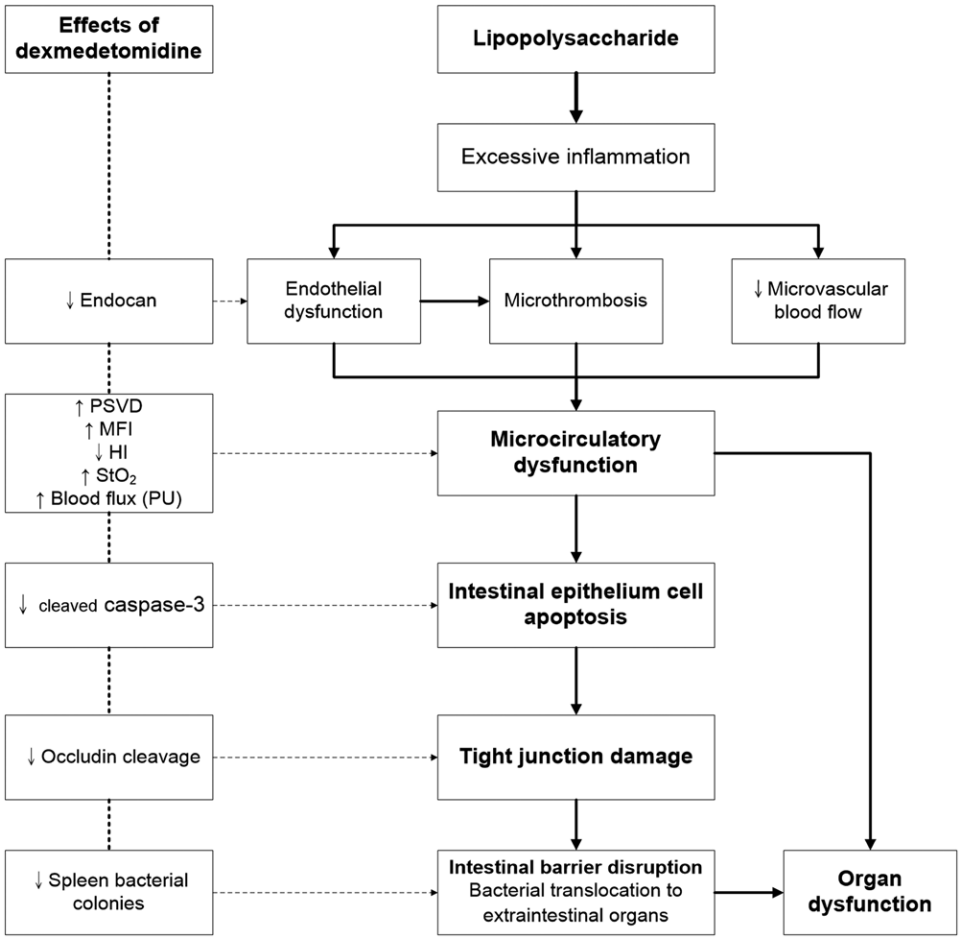

Fig. 7. Summary of hypothesis and results. Blood flux was measured using a full-field laser perfusion imager. Endocan = endothelial cell–specific molecule-1; HI = heterogeneity index; MFI = microvascular flow index; PSVD = perfused small vessel density; PU = perfusion unit; StO_2 = intestine tissue oxygen saturation. ↑ represents a significantly higher value and ↓ represents a significantly lower value in the lipopolysaccharide + dexmedetomidine group than in the lipopolysaccharide group.

were absent in this study. Further investigation with blood gas analysis is required to determine the causes of observed difference in mucosal StO_2 between the lipopolysaccharide and the lipopolysaccharide + dexmedetomidine group.

In conclusion, dexmedetomidine attenuates intestinal microcirculatory alteration and attenuates elevation of endocan levels. In addition, dexmedetomidine reduces epithelial cell death, tight junctional damage, and enteric bacterial translocation to the spleen. We suggest that these benefits of dexmedetomidine in endotoxemic rats may lead to the development of novel therapeutic strategies for septic patients.

Acknowledgments

The authors thank all the participants of National Taiwan University Hospital Center of Microcirculation Medical Research. The authors also thank Roger Lien, B.S.E. (MicroStar Instruments, Taipei, Taiwan, Republic of China), for providing technical assistance during full-field laser perfusion imager examinations.

Research Support

This work was supported, in part, by a grant from the National Science Council (NSC 103-2314-B-002-116), Taipei, Taiwan, Republic of China, and National Taiwan University Hospital (NTUH 103-A125), Taipei, Taiwan, Republic of China.

Competing Interests

The authors declare no competing interests.

Correspondence

Address correspondence to Dr. Yu: Graduate Institutes of Physiology, College of Medicine, National Taiwan University, Suite 1020, No.1, Jen Ai Road, Taipei, Taiwan, Republic of China. lchyu@ntu.edu.tw. 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

1. Coursin DB, Coursin DB, Maccioli GA: Dexmedetomidine. Curr Opin Crit Care 2001; 7:221–6
2. Hoy SM, Keating GM: Dexmedetomidine: A review of its use for sedation in mechanically ventilated patients in an intensive care setting and for procedural sedation. Drugs 2011; 71:1481–501
3. 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
4. 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
5. Gerlach AT, Dasta JF: Dexmedetomidine: An updated review. Ann Pharmacother 2007; 41:245–52
6. Ma D, Hossain M, Rajakumaraswamy N, Arshad M, Sanders RD, Franks NP, Maze M: Dexmedetomidine produces its neuroprotective effect *via* the alpha 2A-adrenoceptor subtype. Eur J Pharmacol 2004; 502:87–97
7. 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
8. Wu RS, Wu KC, Huang CC, Chiang YY, Chen CC, Liao CL, Chu CN, Chung JG: Different cellular responses of dexmedetomidine at infected site and peripheral blood of emdotoxemic BALB/c mice. Environ Toxicol 2015; 30:1416–22
9. 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
10. 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
11. Xu L, Bao H, Si Y, Wang X: Effects of dexmedetomidine on early and late cytokines during polymicrobial sepsis in mice. Inflamm Res 2013; 62:507–14
12. De Backer D, Creteur J, Preiser JC, Dubois MJ, Vincent JL: Microvascular blood flow is altered in patients with sepsis. Am J Respir Crit Care Med 2002; 166:98–104
13. Vallet B: Microthrombosis in sepsis. Minerva Anestesiol 2001; 67:298–301
14. Ince C, Sinaasappel M: Microcirculatory oxygenation and shunting in sepsis and shock. Crit Care Med 1999; 27:1369–77
15. Czabanka M, Peter C, Martin E, Walther A: Microcirculatory endothelial dysfunction during endotoxemia—insights into pathophysiology, pathologic mechanisms and clinical relevance. Curr Vasc Pharmacol 2007; 5:266–75
16. 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; 32:1825–31
17. Vincent JL, De Backer D: Microvascular dysfunction as a cause of organ dysfunction in severe sepsis. Crit Care 2005; 9(suppl 4):S9–12
18. Iba T, Aihara K, Watanabe S, Yanagawa Y, Takemoto M, Yamada A, Yang D: Recombinant thrombomodulin improves the visceral microcirculation by attenuating the leukocyte-endothelial interaction in a rat LPS model. Thromb Res 2013; 131:295–9
19. Iba T, Kidokoro A, Fukunaga M, Nagakari K, Shirahama A, Ida Y: Activated protein C improves the visceral microcirculation by attenuating the leukocyte-endothelial interaction in a rat lipopolysaccharide model. Crit Care Med 2005; 33:368–72
20. Hack CE, Zeerleder S: The endothelium in sepsis: Source of and a target for inflammation. Crit Care Med 2001; 29(7 suppl):S21–7
21. Scherpereel A, Depontieu F, Grigoriu B, Cavestri B, Tsicopoulos A, Gentina T, Jourdain M, Pugin J, Tonnel AB, Lassalle P: Endocan, a new endothelial marker in human sepsis. Crit Care Med 2006; 34:532–7
22. Mihajlovic DM, Lendak DF, Brkic SV, Draskovic BG, Mitic GP, Novakov Mikic AS, Cebovic TN: Endocan is useful biomarker of survival and severity in sepsis. Microvasc Res 2014; 93:92–7
23. Frey L, Kesel K: [Significance of perfusion of the gastrointestinal tract in shock]. Anaesthesist 2000; 49:446–50
24. 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
25. Farquhar I, Martin CM, Lam C, Potter R, Ellis CG, Sibbald WJ: Decreased capillary density *in vivo* in bowel mucosa of rats with normotensive sepsis. J Surg Res 1996; 61:190–6
26. Theuer CJ, Wilson MA, Steeb GD, Garrison RN: Microvascular vasoconstriction and mucosal hypoperfusion of the rat small intestine during bacteremia. Circ Shock 1993; 40:61–8
27. Jiang LY, Zhang M, Zhou TE, Yang ZF, Wen LQ, Chang JX: Changes of the immunological barrier of intestinal mucosa in rats with sepsis. World J Emerg Med 2010; 1:138–43
28. Leaphart CL, Tepas JJ III: The gut is a motor of organ system dysfunction. Surgery 2007; 141:563–9
29. Biffl WL, Moore EE: Splanchnic ischaemia/reperfusion and multiple organ failure. Br J Anaesth 1996; 77:59–70
30. Zhang XY, Liu ZM, Wen SH, Li YS, Li Y, Yao X, Huang WQ, Liu KX: Dexmedetomidine administration before, but not after, ischemia attenuates intestinal injury induced by intestinal ischemia-reperfusion in rats. Anesthesiology 2012; 116:1035–46
31. Wang ZX, Huang CY, Hua YP, Huang WQ, Deng LH, Liu KX: Dexmedetomidine reduces intestinal and hepatic injury after hepatectomy with inflow occlusion under general anaesthesia: A randomized controlled trial. Br J Anaesth 2014; 112:1055–64
32. Huang CY, Hsiao JK, Lu YZ, Lee TC, Yu LC: Anti-apoptotic PI3K/Akt signaling by sodium/glucose transporter 1 reduces epithelial barrier damage and bacterial translocation in intestinal ischemia. Lab Invest 2011; 91:294–309
33. Yu LC, Flynn AN, Turner JR, Buret AG: SGLT-1-mediated glucose uptake protects intestinal epithelial cells against LPS-induced apoptosis and barrier defects: A novel cellular rescue mechanism? FASEB J 2005; 19:1822–35
34. Yeh YC, Wang MJ, Lin CP, Fan SZ, Tsai JC, Sun WZ, Ko WJ: Enoxaparin sodium prevents intestinal microcirculatory dysfunction in endotoxemic rats. Crit Care 2012; 16:R59
35. Yeh YC, Ko WJ, Chan KC, Fan SZ, Tsai JC, Cheng YJ, Sun WZ: Effects of eritoran tetrasodium, a toll-like receptor 4 antagonist, on intestinal microcirculation in endotoxemic rats. Shock 2012; 37:556–61
36. Lu YZ, Wu CC, Huang YC, Huang CY, Yang CY, Lee TC, Chen CF, Yu LC: Neutrophil priming by hypoxic preconditioning protects against epithelial barrier damage and enteric bacterial translocation in intestinal ischemia/reperfusion. Lab Invest 2012; 92:783–96
37. De Backer D, Hollenberg S, Boerma C, Goedhart P, Buchele G, Ospina-Tascon G, Dobbe I, Ince C: How to evaluate the microcirculation: Report of a round table conference. Crit Care 2007; 11: R101
38. Wu LL, Chiu HD, Peng WH, Lin BR, Lu KS, Lu YZ, Yu LC: Epithelial inducible nitric oxide synthase causes bacterial translocation by impairment of enterocytic tight junctions

via intracellular signals of Rho-associated kinase and protein kinase C zeta. Crit Care Med 2011; 39:2087–98

39. Yu LC, Yang PC, Berin MC, Di Leo V, Conrad DH, McKay DM, Satoskar AR, Perdue MH: Enhanced transepithelial antigen transport in intestine of allergic mice is mediated by IgE/CD23 and regulated by interleukin-4. Gastroenterology 2001; 121:370–81
40. Chen TL, Chen S, Wu HW, Lee TC, Lu YZ, Wu LL, Ni YH, Sun CH, Yu WH, Buret AG, Yu LC: Persistent gut barrier damage and commensal bacterial influx following eradication of Giardia infection in mice. Gut Pathog 2013; 5:26
41. Yu LC, Montagnac G, Yang PC, Conrad DH, Benmerah A, Perdue MH: Intestinal epithelial CD23 mediates enhanced antigen transport in allergy: Evidence for novel splice forms. Am J Physiol Gastrointest Liver Physiol 2003; 285:G223–34
42. Wu LL, Peng WH, Kuo WT, Huang CY, Ni YH, Lu KS, Turner JR, Yu LC: Commensal bacterial endocytosis in epithelial cells is dependent on myosin light chain kinase-activated brush border fanning by interferon-γ. Am J Pathol 2014; 184:2260–74
43. Kuo WT, Lee TC, Yang HY, Chen CY, Au YC, Lu YZ, Wu LL, Wei SC, Ni YH, Lin BR, Chen Y, Tsai YH, Kung JT, Sheu F, Lin LW, Yu LC: LPS receptor subunits have antagonistic roles in epithelial apoptosis and colonic carcinogenesis. Cell Death Differ 2015; 22:1590–604
44. Yu LC, Shih YA, Wu LL, Lin YD, Kuo WT, Peng WH, Lu KS, Wei SC, Turner JR, Ni YH: Enteric dysbiosis promotes antibiotic-resistant bacterial infection: Systemic dissemination of resistant and commensal bacteria through epithelial transcytosis. Am J Physiol Gastrointest Liver Physiol 2014; 307:G824–35
45. Levi M, van der Poll T: Inflammation and coagulation. Crit Care Med 2010; 38:S26–34
46. Webster NR: Inflammation and the coagulation system. Br J Anaesth 2002; 89:216–20
47. Joyce DE, Nelson DR, Grinnell BW: Leukocyte and endothelial cell interactions in sepsis: Relevance of the protein C pathway. Crit Care Med 2004; 32(5 suppl):S280–6
48. van Griensven M, Probst C, Müller K, Hoevel P, Pape HC: Leukocyte-endothelial interactions *via* ICAM-1 are detrimental in polymicrobial sepsis. Shock 2006; 25:254–9
49. Iba T, Okamoto K, Kawasaki S, Nakarai E, Miyasho T: Effect of hemoperfusion using polymyxin B-immobilized fibers on non-shock rat sepsis model. J Surg Res 2011; 171:755–61
50. Vincent JL: Microvascular endothelial dysfunction: A renewed appreciation of sepsis pathophysiology. Crit Care 2001; 5:S1–5
51. Liang Y, Li X, Zhang X, Li Z, Wang L, Sun Y, Liu Z, Ma X: Elevated levels of plasma TNF-α are associated with microvascular endothelial dysfunction in patients with sepsis through activating the NF-κB and p38 mitogen-activated protein kinase in endothelial cells. Shock 2014; 41:275–81
52. Miranda ML, Balarini MM, Bouskela E: Dexmedetomidine attenuates the microcirculatory derangements evoked by experimental sepsis. Anesthesiology 2015; 122:619–30
53. Gets J, Monroy FP: Effects of alpha- and beta-adrenergic agonists on Toxoplasma gondii infection in murine macrophages. J Parasitol 2005; 91:193–5
54. Morelli A, Donati A, Ertmer C, Rehberg S, Kampmeier T, Orecchioni A, D'Egidio A, Cecchini V, Landoni G, Pietropaoli P, Westphal M, Venditti M, Mebazaa A, Singer M: Microvascular effects of heart rate control with esmolol in patients with septic shock: A pilot study. Crit Care Med 2013; 41:2162–8
55. Martin E, Ramsay G, Mantz J, Sum-Ping ST: The role of the alpha2-adrenoceptor agonist dexmedetomidine in postsurgical sedation in the intensive care unit. J Intensive Care Med 2003; 18:29–41
56. Jakob SM, Ruokonen E, Grounds RM, Sarapohja T, Garratt C, Pocock SJ, Bratty JR, Takala J; Dexmedetomidine for Long-Term Sedation Investigators: Dexmedetomidine *vs* midazolam or propofol for sedation during prolonged mechanical ventilation: Two randomized controlled trials. JAMA 2012; 307:1151–60
57. Ozaki M, Takeda J, Tanaka K, Shiokawa Y, Nishi S, Matsuda K, Doi M, Kakihana Y, Fujino Y, Takinami M, Kawai M: Safety and efficacy of dexmedetomidine for long-term sedation in critically ill patients. J Anesth 2014; 28:38–50
58. 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